ASPECTS OF OLFACTORY COMMUNICATION IN THE DWARF HAMSTER (*PHODOPUS SUNGORUS*) AND THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*)

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ASPECTS OF OLFACTORY COMMUNICATION IN THE DWARF HAMSTER (PHODOPUS SUNGORUS) AND THE NAKED MOLE-RAT (HETEROCEPHALUS GLABER)

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Olfactory communication is an understudied phenomenon, likely due to human bias against this modality of communication. I expanded our knowledge of olfactory communication by testing the following hypotheses: self-grooming is a form of olfactory communication in the dwarf hamster *Phodopus sungorus*; naked mole-rats (*Heterocephalus glaber*) are capable of social-dominance-based discrimination of odor; and rolling behaviors of naked mole-rats function as a form of scent-marking.

Self-grooming is ubiquitous among mammals, yet our knowledge of the functions of this behavior beyond cleaning the body surface is limited. Dwarf hamsters were exposed to cotton nesting material scented by same-sex and opposite-sex conspecifics, as well as an unscented control. As predicted, subjects self-groomed more in response to conspecific odor than unscented controls, an important first step in demonstrating communication. Furthermore, self-grooming in response to opposite-sex odor was higher than same-sex odor and unscented controls, which did not differ from each other, suggesting that its communicative function is to attract a mate.

Naked mole-rats live in underground eusocial colonies in which social dominance plays important roles in their reproductive division of labor, cooperation, mate choice, and access to food. Three colonies were tested using a T-choice apparatus in which each stimulus arm contained the whole-body odor of one of two fellow colony members that differed in dominance rank. Subjects were tested with several pairs of stimulus odor donors that varied in dominance status, sex, breeding status, and body weight, and subjects were analyzed separately according to sex, breeding status, absolute dominance rank, and relative dominance rank. The overall results were consistent: naked mole-rats mostly preferred to enter the arm containing dominant odor.

Naked mole-rats perform rolling behaviors in which an individual's dorsum contacts the floor, and these behaviors may function in scent-marking. A plastic tube in each of three test colony tunnel systems was replaced on alternating days with a clean tube, to stimulate scent-marking, or a "dirty" tube that had been part of the colony tunnel system for 24 h prior. For both sexes, rolling behaviors were more frequent in the clean than the dirty stimulus tube condition within 2.75 h of tube replacement.

BIOGRAPHICAL SKETCH

Frank Robert Castelli has a deep passion for nature and the scientific method. He is particularly interested in animal social behavior and evolution. His academic interests have been shaped by many influential educators throughout his life.

Frank was born and raised on the border of Brooklyn and Queens, NY by Robert Castelli and Kim Castelli along with his younger brother, Robert. He developed an interest in science in elementary school and became aware that becoming a scientist was a possible occupation in large part due to his love for the Ghostbusters, particularly the character Egon Spengler. He attended high school on Long Island where he developed a more specific interest in biology.

During the summer before his senior year of high school he worked in the laboratory of Ivan Chace at SUNY Stony Brook studying winner and loser effects in cichlid fish. He continued to work with Dr. Chase on a project investigating ant foraging and recruitment to food sources which won Honorable Mention at the Long Island Science Congress.

Soon after high school graduation, he was living in Nevada collecting stickleback fossils in the desert with SUNY Stony Brook researcher Michael Bell. In the fall he matriculated into the Honors College of SUNY Stony brook and worked on fossilized bird regurgitations in Dr. Bell's lab. During his sophomore year, he worked in the lab of Douglas Futuyma rearing beetle and moth larva on plants with photosynthetic mutations. In the following summer, Frank took a rainforest biology course in Costa Rica and a primate biology course in Nicaragua in which he studied

howler monkeys.

In the fall of 2002, Frank transferred to the College of Agriculture and Life Sciences at Cornell University where he first studied naked mole-rats in the lab of Paul Sherman. The following summer, Frank studied the Utah prairie dog with John Hoogland of the University of Maryland, College Park. Frank graduated *magna cum laude* with a Bachelor of Science in Biology and helped the Cornell Men's Fencing Club win the national club championships. After graduating from Cornell, he continued to work with naked mole-rats in Dr. Sherman's lab and did a small project on tailless whip scorpions with Linda Rayor in the Entomology Department. Dr. Rayor taught him to become a passionate outreach speaker of spider biology which helped him overcome his arachnophobia.

Frank went on to study prairie voles in the lab of Nancy Solomon at Miami University in Ohio where he learned to trap rodents in the field and genotype in the laboratory. He returned to Cornell for graduate school as a Sage Fellow and joined the lab of Robert Johnston where he earned a Masters in Psychology and a Psychology Department Teaching Award on his way to finishing his Ph. D. in Behavioral Evolutionary Neuroscience.

Subsequently, Frank has joined the lab of Catherine Marler in the Psychology Department of the University of Wisconsin-Madison studying the social behavior of California mice.

In memory of Robert E. Johnston, supportive mentor and kindred spirit.

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CHAPTER 1

INTRODUCTION

Olfactory communication is an understudied phenomenon of the natural world, likely due to bias from the fact that humans do not rely on this modality of communication. Nisbett (1977, p. 30) writes in his biography of Konrad Lorenz: "Apart from man, most other animals think through their noses. Lorenz delights in a remark by Sir Julian Huxley that if we, too, were olfactory animals there would be no bird watchers, but in their place we would have mammal smelling societies." Alas, it seems that there are no mammal smelling societies and the natural inclination for humans to focus scientific study on what is more easily perceived can partly explain why our understanding of olfactory communication is quite limited in comparison to other phenomena.

Nevertheless, a reliance on olfactory communication is widespread in the animal kingdom and a greater understanding of the proximate and ultimate causes of animal behavior would be greatly served by investigating this phenomenon. There are many avenues of investigation that students of olfactory communication could pursue and many potential study organisms. The goal of this dissertation is to expand our knowledge of olfactory communication by asking two broad questions: "What behaviors are involved in communicating with odors?" and "what information is communicated via odor?" These questions are explored using two rodent species, a species of dwarf hamster (*Phodopus sungorus*) and the naked mole-rat

(*Heterocephalus glaber*). In general, rodents are excellent study organisms to investigate olfactory communication due to their great reliance on this mode of communication. Specifically, these two species provide variation in the extent of reliance on olfaction as well as the level of their social interactions. The naked mole-rat is fossorial, nearly blind, and lives in colonies of many individuals of either sex (Jarvis & Sherman, 2002). The *Phodopus sungorus* dwarf hamster, in contrast, is a semi-fossorial species with better vision and lives a mostly solitary lifestyle except when reproducing (Calderone & Jacobs, 1999; Wynne-Edwards, 2003).

Aside from the human bias against the cognizance of olfactory communication in general, bias may also contribute to a failure of imagination when it comes to answering the first broad question explored in this dissertation: "what behaviors are involved in communicating with odors?" Self-grooming behavior was labeled as such based on the perception of its apparent function, to clean the body surface. However, in *Chapter 2* I test the hypothesis that self-grooming functions in olfactory communication in the *Phodopus sungours* dwarf hamster, possibly by dispersing volatile chemicals. Similarly, a rolling behavior of naked mole-rats was labeled as a turning behavior that functions to reverse orientation and change the direction of movement in a narrow subterranean tunnel (Lacey, Alexander, Braude, Sherman, & Jarvis, 1991). However, in *Chapter 4*, I test the hypothesis that rolling behaviors in naked mole-rats function as a form of scent-marking. The second broad question investigated in this dissertation is, "what information is communicated via odor?" In *Chapter 3*, I test the hypothesis that naked mole-rats are able to discriminate between

the odors of fellow colony members based on the dominance of odor donors.

Self-grooming is a widespread behavior across taxa and ubiquitous among mammals, yet our knowledge of the functions of this behavior beyond cleaning the body surface is limited. While it seems likely that many behaviors labeled as "grooming" originally provided and continue to provide fitness benefits by removing foreign substances and parasites from the body surface, these motor patterns may have conferred additional benefits in other contexts (Spruijt, van Hooff, & Gispen, 1992). The behavioral actions of self-grooming may facilitate the release of volatile chemicals into the air (Ferkin, Sorokin, & Johnston, 1996). Regardless of the precise mechanism, self-grooming may function in olfactory communication (e.g., Ferkin, Leonard, Heath, & Paz-y-Miño C., 2001; Ferkin et al., 1996). Early steps in determining if a behavior has a communicative function are to see if it is elicited at all by social stimuli and to see if the amount of elicitation is influenced by the type of the social stimulus. *Chapter 2* describes a repeated measures design in which male and female *Phodopus sungorus* dwarf hamsters were exposed to cotton nesting material scented by same-sex and opposite-sex conspecifics, as well as an unscented control, to look for differences in self-grooming.

Naked mole-rats are atypical among mammals in their extreme form of eusocial cooperative breeding, and social dominance plays important roles in their reproductive division of labor, cooperation, mate choice, and access to food (Clarke & Faulkes, 1997, 1998, Jarvis, 1981, 1991; Schieffelin & Sherman, 1995). Therefore, it is likely that natural selection has favored the evolution of mechanisms for individuals

to discriminate between fellow colony members based on dominance. Since naked mole-rats are nearly blind, live underground, and are highly attuned to odor (Brett, 1991; Hetling et al., 2005; O'Riain & Jarvis, 1997), it is also likely that the use of body odor is one such mechanism for discrimination based on dominance. *Chapter 3* describes a repeated measures design in which subjects of three colonies of naked mole-rats were tested using a T-choice apparatus in which each stimulus arm contained the whole-body odor of one of two fellow colony members that differed in dominance rank and choice was measured by stimulus arm entry. Subjects were tested with several pairs of stimulus odor donors that varied in dominance status, sex, breeding status, and body weight, and subjects were analyzed separately according to sex, breeding status, absolute dominance rank, and relative dominance rank.

Naked mole-rats live in vast networks of subterranean tunnels and chambers (Brett, 1991) and perform rolling behaviors in which an individual's dorsal surface makes contact with the floor of a tunnel (e.g., Lacey et al., 1991). In some forms of rolling behaviors, the individual's body reverses orientation in the tunnel while in others it is maintained. Those rolling behaviors that reverse orientation may have originally evolved to function as a way to change the direction of movement. However, several observations suggest that these and rolling behaviors that maintain orientation may additionally or alternatively function in olfactory communication as a form of scent-marking. *Chapter 4* describes a study in which a plastic tube in each of three colonies was replaced on alternating days with a clean tube or a "dirty" tube that had been part of the colony tunnel system for 24 h prior, and rolling behaviors and

upright turns were scored for 2.75 h each day for 20 days.

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CHAPTER 2

SELF-GROOMING IN RESPONSE TO CONSPECIFIC ODORS IN A DWARF HAMSTER, PHODOPUS SUNGORUS: EVIDENCE FOR OLFACTORY COMMUNICATION

ABSTRACT

Self-grooming is a widespread behavior across taxa and ubiquitous among mammals, yet our knowledge of the functions of this behavior beyond simply cleaning the body surface is limited. Another possible function of self-grooming is to facilitate communication with conspecifics via the spreading of odor, a function that has been systematically investigated in only a few species. This study tests the hypothesis that self-grooming functions in olfactory communication in *Phodopus sungorus* dwarf hamsters, a species for which much is known about their olfactory communication. In a repeated measures design, male and female hamsters were exposed to cotton nesting material scented by same-sex and opposite-sex conspecifics, as well as an unscented control, to look for differences in self-grooming response, an important first step in demonstrating communication. As predicted, subjects self-groomed more in response to conspecific odor than unscented controls. Specifically, self-grooming in response to opposite-sex odor was higher than same-sex odor and unscented controls, which did not differ from each other, suggesting that a communicative function for self-grooming is to attract a mate. This pattern of self-grooming was similar when the grooming of different body parts was analyzed separately. Consistent with these

findings, subjects spent more time investigating opposite-sex odor than same-sex odor or unscented controls, and subjects also showed a greater interest in communicating with the opposite-sex through scent-marking. This study supports the hypothesis that self-grooming functions in olfactory communication in *Phodopus sungorus* and that this communicative function is likely to attract a mate. Additional studies are needed to examine this phenomenon in greater detail.

INTRODUCTION

Grooming one's own body (self-grooming or auto-grooming) is a widespread behavior across taxa and ubiquitous among mammals, yet our knowledge of the functions of this behavior beyond cleaning the body surface is limited. While it seems likely that many behaviors labeled as "grooming" originally provided and continue to provide fitness benefits by removing foreign substances and parasites from the body surface, these motor patterns may have conferred additional benefits in other contexts (reviewed by Spruijt, van Hooff, & Gispen, 1992). For example, grooming behaviors may also function to relieve anxiety, to cool the body via spreading saliva that evaporates, to warm the body via spreading gland exudate with radiation-absorbing pigments, to heal wounds via licking them with saliva containing antimicrobial properties, and to release tension by satisfying an urge to behave in some other way that is being prevented, a.k.a. "displacement grooming" (Spruijt et al., 1992).

Another possible function of self-grooming, and the focus of this study, is communication between conspecifics. In highly visually-oriented species, such as

primates, self-grooming may be used to communicate to others a relaxed or indifferent state, or used as a way to avoid interaction (Spruijt et al., 1992). In less visually-oriented species, like rodents, self-grooming may play important functional roles in olfactory communication.

One mechanism by which grooming may enhance olfactory communication is by increasing the quantity of volatile odors and semiochemicals released into the air. This may be achieved in several ways including the following: increasing the amount of semiochemical-containing exudate that is drawn out of glands; increasing the surface area of the body over which these semiochemicals are spread; and mixing semiochemicals with saliva to facilitate evaporation (Ferkin, Sorokin, & Johnston, 1996; Shanas & Terkel, 1995, 1997). Additionally, grooming with the mouth deposits saliva which itself can have communicative properties (e.g., Lai & Johnston, 1994) and salivary enzymes may react with skin microflora to release additional volatiles that may play a role in communication (Schaal & Al Aïn, 2014). Regardless of the precise mechanism, self-grooming may enhance olfactory communication. For example, female prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*) of both sexes showed a preference for odor from opposite sex individuals that groomed at high rates versus low rates (Ferkin, Leonard, Heath, & Paz-y-Miño C., 2001; Ferkin et al., 1996).

The information carried by this signal, and thus the communicative functions of self-grooming, may differ between species and within species, and it may depend on the sex of the groomer, the sex of the target audience, and the context of grooming.

Whether or not the intended audience is of the same or opposite sex as the groomer, the signal may be agonistic or sociable.

Same-sex communication is often associated with intrasexual competition for resources or potential mates. Therefore, self-grooming may function in communicating with a same-sex rival by sending information about territorial ownership, mate possession, dominance, and fighting prowess. Providing such information to rivals from a distance can potentially preclude wasting energy and risking injury in a fight. In the wild, territorial male California ground squirrels (*Otospermophilus beecheyi*) commonly self-groom on the boundaries of their territories, and high intensity fights with other males are commonly punctuated by sudden bouts of self-grooming that are often followed by scent-marking (Bursten, Berridge, & Owings, 2000; Durant, Dole, & Fisler, 1988). Interactions between two males were also less likely to escalate into a fight when they included bouts of self-grooming (Bursten et al., 2000). While Bursten and colleagues (2000) suggest that self-grooming between males of this species functions as a visual agonistic signal, this does not preclude a function in olfactory communication. Male prairie voles and male meadow voles self-groom more in response to same-sex odor than unscented controls (Ferkin et al., 2001; Leonard & Ferkin, 2005). Male prairie voles also self-groom more in response to same-sex odor than opposite-sex odor while male meadow voles preferentially self-groom in response to females (Ferkin et al., 2001; Leonard & Ferkin, 2005). Since prairie voles are socially monogamous and form pair-bonds (Getz, Carter, & Gavish, 1981) while male meadow voles are not (Madison, 1980),

Ferkin and colleagues (2001) suggest that a male prairie voles may be particularly motivated to communicate with same-sex conspecifics as a form of mate guarding. Female meadow voles housed under a long photoperiod, simulating the breeding season, also self-groom more in response to same-sex odor than unscented controls (Leonard & Ferkin, 2005). Since female meadow voles are territorial and aggressive with each other during the breeding season (Ferkin & Seamon, 1987; Madison, 1980), self-grooming may function to reaffirm ownership of a territory (Ferkin & Leonard, 2010).

In addition to agonistic functions, communicating with the same-sex via self-grooming may have sociable functions. For example, during the non-breeding winter months, female meadow voles preferentially approach the odor of other females, reduce same-sex aggression, and commonly nest together in mixed-sex groups for thermoregulation (Ferkin & Seamon, 1987; Madison, FitzGerald, & McShea, 1984; Madison & McShea, 1987). When reared in captivity under short photoperiods simulating the winter months, females preferentially self-groomed in response to same-sex odor over unscented and heterospecific controls, suggesting a function in the facilitation of communal nesting (Ferkin & Leonard, 2010; Leonard & Ferkin, 2005).

Self-grooming may also function in communication with the opposite sex, most obviously for the purpose of attracting a mate. By increasing the volatility of their odors, self-groomers may be able to send information to a potential mate about their sexual identity, willingness to mate, and quality, from a distance, which may preclude wasting effort, incurring opportunity costs, and risking injury from leery

potential mates. Meadow voles and prairie voles of both sexes housed under long photoperiods, simulating the breeding season, self-groomed at higher rates in response to odor from unrelated opposite-sex conspecifics than unscented controls (Ferkin et al., 2001, 1996; Leonard & Ferkin, 2005; Paz-y-Miño C., Leonard, Ferkin, & Trimble, 2002). In blind mole-rats (*Spalax ehrenbergi*), self-grooming spreads Harderian gland secretion over the body, and aggression by females towards males significantly decreases immediately after males self-groom (Shanas & Terkel, 1997). The mitigation of aggression between potential mates is a necessary early step in courtship, especially in species that are solitary and highly aggressive like the blind mole-rat (Shanas & Terkel, 1997). Additionally, blind mole-rats of both sexes spend more time investigating the odor of Harderian gland secretions than control odors, suggesting that self-grooming may also release odors that allow for the evaluation of a potential mate (Shanas & Terkel, 1997). Self-grooming to spread Harderian gland secretion may have similar effects in other rodents, including golden hamsters (*Mesocricetus auratus*) and Mongolian gerbils (*Meriones unguiculatus*) (Payne, 1977; Thiessen & Harriman, 1986; Thiessen, Clency, & Goodwin, 1976).

The function of self-grooming to attract a mate is also supported by observations of self-grooming behavior that reflect an individual's willingness to mate. An individual's willingness to mate can be influenced by its internal state. Consistent with the finding that high titers of testosterone are necessary for male meadow voles to prefer the odor of females (Leonard & Ferkin, 1999), gonadectomized male and female meadow voles spent less time self-grooming in

response to opposite-sex odor than did gonadectomized voles that received replacement sex steroid hormones (Ferkin, 2006). Photoperiod can also affect an individual's internal state. Female meadow voles spent more time self-grooming in response to male odor than female odor when housed under long photoperiods, while those housed under short photoperiods showed no preference (Leonard & Ferkin, 2005). In long-tailed ground squirrels (*Urocitellus undulates*) and Columbian ground squirrels (*Urocitellus columbianus*), courtship bouts and chases of females by males in the wild were "characteristically" punctuated with abrupt and repeated bursts of self-grooming that peaked during the long photoperiod of the reproductive season (Steiner, 1973).

An individual's willingness to mate can also be influenced by the attractiveness of a potential mate and this may also be reflected by self-grooming behavior. Male meadow voles spent significantly more time self-grooming in response to the odors of intact females in postpartum estrus than they did to ovariectomized females, and female meadow voles spent significantly more time self-grooming in response to the odors of orchiectomized males receiving testosterone replacement than they did to orchiectomized males that did not (Ferkin, 2006). This is consistent with the finding that males must have high titers of testosterone in order for females to spend more time investigating their odors (Ferkin, Sorokin, & Johnston, 1997). Male and female meadow voles housed under long photoperiods self-groomed more in response to odor from opposite-sex conspecifics that were also reared under long photoperiods as compared to opposite-sex conspecifics reared under short

photoperiods (Leonard & Ferkin, 2005). Matching their preferences to outbreed (Bollinger, Harper, Kramer, & Barrett, 1991; McGuire & Getz, 1981), meadow voles and prairie voles of both sexes spent more time self-grooming in response to the odors of opposite-sex non-siblings than to the odors of opposite-sex siblings or unscented controls (Paz-y-Miño C. et al., 2002). Meadow voles prefer to eat plants with high protein content, which improves their fitness (Bergeron & Joudoin 1989), and potential mates that have eaten food with higher protein content are presumably better at competing for resources and may have good genes that promote longevity of offspring. Female meadow voles spent more time investigating the odor of males that were fed high protein diets (Ferkin, Sorokin, Johnston, & Lee, 1997) and self-groomed more in response to high-protein-diet males (Hobbs, Aven, & Ferkin, 2008). Male root voles (*Microtus oeconomus*) spent more time investigating and self-grooming in response to odors of lactating females than non-lactating females (Yu, Yue, Sun, & Zhao, 2010).

In addition to attracting a mate, communicating with the opposite-sex via self-grooming may also have agonistic functions, particularly for species in which both sexes are solitary or territorial. For example, in the solitary and highly aggressive blind mole-rat, males and females were equally likely to establish dominance in an opposite-sex dyadic interaction, and aggression while establishing dominance positively correlated with self-grooming (Shanas & Terkel, 1995). Moreover, the subordinate of the pair showed more self-grooming than the dominant, suggesting that self-grooming may communicate subordination and unwillingness to continue fighting (Shanas &

Terkel, 1995), further supported by a decrease in aggression by females towards males immediately after males self-groom (Shanas & Terkel, 1997).

Despite the mounting evidence in support of the existence of functions of self-grooming in olfactory communication, there have been disproportionally few controlled studies of this incredibly widespread behavior, and only a paucity of species have been tested. At minimum, we still do not know the prevalence of these functions. Knowledge of these functions in additional species is necessary to make broader conclusions about their evolutionary origins and phylogenetic relationships. Furthermore, self-grooming is a potentially valuable behavior for studying the evolution of olfactory communication since its communicative functions are likely to vary between species and between sexes and contexts within species.

Phodopus sungorus is one of the three species of dwarf hamsters (*Phodopus campbelli* and *Phodopus roborovskii* being the other two), all native to Central Asia (Ross, 1994, 1995, 1998), and it is an excellent species for expanding our knowledge of the functions of self-grooming in olfactory communication. Common names of *Phodopus* species have been inconsistently used in the literature (Steinlechner, 1998), so I will refer to them by their scientific names. *Phodopus* species are amenable for study of olfactory communication in the field (Wynne-Edwards, Surov, & Telitzina, 1992, 1999) and laboratory, and they have several different sources of odors with which they communicate different or overlapping information, including urine, feces, Harderian glands, and vaginal glands. In addition to these commonly studied odors in rodents, *Phodopus* have evolved specialized sebaceous mid-ventral glands and, in two

species (*Phodopus sungorus* and *Phodopus campbelli*), a unique pair of supplementary sacculi at the openings of cheek pouches that produce a creamy odorous substance (Feoktistova, 1994; Feoktistova & Meschersky, 1999; Reasner & Johnston, 1987). Additional odor sources have been suggested, including saliva, the feet (forepaws and hindpaws), and secretions from the scrotum and behind the ears (Lai & Johnston, 1994; Litvinova & Vasilieva, 2004a; Wynne-Edwards, 2003; Wynne-Edwards et al., 1992). In *Phodopus sungorus*, males have been shown to distinguish on the basis sex between the odor of urine, feces, mid-ventral gland secretion, sacculus secretion, and soiled wood shavings that are a mixture of different odor sources (Feoktistova, 1994; Feoktistova & Meschersky, 1999). Many of their odors sources may also be used to discriminate between individuals (Lai & Johnston, 1994; Litvinova & Vasilieva, 2004a; Vasilieva & Sokolov, 1994). The odors of the sacculi are also believed to play a role in marking and retrieving cached food (Apfelbach, Schmidt, & Vasilieva, 2001). *Phodopus* mid-ventral glands are sexually dimorphic (larger in males), used in scent-marking for communication between and within sexes, and have distinctive hairs that appear to be specialized for distributing secretion from the gland to substrate (Feoktistova, 1994; Heisler, 1984a, 1984b; Reasner & Johnston, 1987; Wynne-Edwards, 2003; Wynne-Edwards & Lisk, 1987b, 1987a, 1988; Wynne-Edwards et al., 1992).

Self-grooming is believed to be an important component of olfactory communication in *Phodopus* based on observations in the field (Wynne-Edwards, 2003; Wynne-Edwards et al., 1992). Extensive focal observations of two *Phodopus*

campbelli females in the field revealed that bouts of self-grooming were associated with scent-marking and that bouts of self-grooming were significantly more likely to occur immediately prior to scent-marking than after, suggesting a communicative function of self-grooming (Wynne-Edwards et al., 1992). Wynne-Edwards and colleagues (Wynne-Edwards, 2003; Wynne-Edwards et al., 1992) proposed that by self-grooming, *Phodopus* hamsters spread their various odor sources around the surface of their body, and that the characteristically dense fur of their forepaws and hindpaws may function in territorial navigation by absorbing these odors and distributing them on the ground during locomotion. The possible importance of spreading one's own odor over the body via self-grooming was nicely demonstrated in a laboratory study in which *Phodopus sungorus* males discriminated between the odors of other males that had their fur washed with detergents to remove odors. When stimulus males had their mid-ventral glands and sacculi surgically removed, it took eight days after washing before subjects could distinguish between individuals using fur odor, while it only took two days with males that had only undergone sham surgery (Litvinova & Vasilieva, 2004b). The implication is that without the prominent secretions from the mid-ventral gland and sacculi to spread over their body via self-grooming, it took much longer for other sources of odor to build up that enabled subjects to distinguish individuals.

Only one study that I am aware of has looked at self-grooming in *Phodopus sungorus* under controlled laboratory conditions and the methodology differs in many ways from the current study. Feoktistova (1994) presented ten sexually-experienced

Phodopus sungorus males with four different odor sources from same-sex and opposite-sex odor donors and measured two categories of self-grooming behavior. She found that males self-groomed (over the ears and around the body, N.Y. Feoktistova, personal communication, 20 September 2014) more in an arena containing a small sample of mid-ventral gland secretion from same-sex odor donors than they did in a clean arena, and there was no difference in self-grooming between opposite-sex and same-sex conditions or opposite-sex and clean conditions. Furthermore, there were no differences in either of two types of self-grooming (rubbing face with forepaws, or over the ears and around the body, N.Y. Feoktistova, personal communication, 20 September 2014) in response to urine, sacculus secretion, or soiled bedding in either same-sex, opposite-sex, or clean stimulus conditions (Feoktistova, 1994). More studies are needed to examine the communicative functions of self-grooming in rodents.

The primary goal of the current study was to test the hypothesis that self-grooming functions in olfactory communication in the dwarf hamster *Phodopus sungorus* by systematically examining a critical initial component of communication, the differential production of a signal in response to potential recipients (targeting intended receivers). In a repeated measures design, male and female hamsters were exposed to cotton nesting material scented by same-sex and opposite-sex conspecifics, as well as an unscented control, to look for differences in self-grooming and other behaviors. In addition to thoroughly studying the communicative functions of self-grooming in additional species, there is also a need for examination of the

phenomenon in greater detail. Therefore, the current study also separates self-grooming behavior into the different body parts targeted for greater insight. It was predicted that conspecific odors would elicit higher amounts of investigation, self-grooming, and scent-marking than unscented controls, but no particular pattern was predicted when comparing sexes.

METHODS

Animal origins and housing

Seventy one *Phodopus sungorus* dwarf hamsters were used in this study. Hamsters were born and reared in the vivaria of Dr. Ned J. Place (N = 61, College of Veterinary Medicine, Cornell University, Ithaca, NY) or Dr. Leann Kanda (N = 10; Department of Biology, Ithaca College, Ithaca, NY). Dr. Kanda's hamsters were derived from hamsters obtained from Dr. Place and Dr. Place's hamsters were originally derived from wild-bred stock obtained from Dr. Katherine Wynne-Edwards (Queens University, Kingston, Ontario, Canada). The hamsters used in this study were reared under a variety of conditions, but all were provided food and water *ad libitum* and weaned between 18-23 d after birth. Some were raised by both parents, others by mom alone. Weaned animals were either housed alone or with one or more same-sex or opposite-sex siblings. The light cycle (hours of light [L]: hours of dark [D]) of rearing was either 8L:16D, 16L:8D, or 14L:10D. Such a diversity of rearing history is likely more representative of the variation that exists in nature than if all animals were reared under identical conditions. Hamsters of different backgrounds were spread

across the different stimulus conditions of this study to avoid clustering.

All hamsters were transferred from their original laboratories to the same room within the vivarium of the Department of Psychology at Cornell University (Ithaca, NY) where they experienced the same housing conditions for at least two weeks prior to conducting trials and in most cases for months prior. Hamsters were singly housed in polycarbonate cages (L x W x H; 48.3 x 26.7 x 15.6 cm) with stainless steel wire bar lids containing wood litter (Sani-chips, P.J. Murphy Forest Products Corp., Montville, NJ), a cotton Nestlet (Ancare Corp., Bellmore, NY) for constructing a nest, and a wood gnawing block (3.81 x 3.81 x 3.81 cm; #K3512, Bio-Serv, Inc., Flemington, NJ) for environmental enrichment. In the weeks prior to the experiment, cotton Nestlets were substituted with 3 cotton balls (Wegman's Food Markets, Inc., Rochester, NY) to provide previous exposure to the stimulus substrate. Water and food (Prolab RMH 1000, LabDiet, PMI Nutrition Inernational, LLC, Brentwood, MO) were provided *ad libitum*. The colony room was set to 22.2°C and 40%RH. White ceiling lights simulated sunrise and sunset with the onset of dim light at 1830 (Eastern Standard Time; EST), full light at 1915 EST, dim light again at 0845 EST, and full darkness at 0915 EST, for a total lights cycle of 14.75L:9.25D.

Subjects, scent donors,and trial procedures

Each of 60 subjects (N = 30 males, N = 30 females) were tested in three separate trials for their behavioral response to cotton scented by an opposite-sex conspecific, cotton scented by a same-sex conspecific, or unscented clean cotton

(control). The ages of female subjects ranged from 136-332 d (media*N* = 217 d) and the ages of male subjects ranged from 137-452 d (media*N* = 231 d). Hamster-scented cotton was created by providing scent donors with three cotton balls for at least three days prior to use. The cotton was used by donors to construct nests and thus likely acquired odor from various bodily sources, including urine, feces (pellets were visible), saliva (cotton is often stuffed into cheek pouches prior to nest construction), the ano-genital region, the mid-ventral gland, and other scent glands. Clean cotton balls were pulled apart and placed in a clean empty cage that contained all the possible abiotic odor sources that a cage with an odor donor would contain, i.e., food, water, litter, and a wood block.

Sixty-nine hamsters were used as stimulus scent donors, 58 of which were also subjects. In each trial, donors were unrelated and unfamiliar to subjects. The number of hamsters that were used as donors in one trial, two trials, or three trials was 22, 43, and four. When used in more than one trial, donors were mostly balanced across opposite-sex and same-sex trials. The ages of donors were as follows: female donors for opposite-sex stimulus trails ranged from 136-324 d (media*N* = 232 d), male donors for opposite-sex stimulus trials ranged from 164-360 d (media*N* = 227.5 d), female donors for same-sex stimulus trials ranged from 145-352 d (media*N* = 237 d), male donors for same-sex stimulus trials ranged from 164-468 d (media*N* = 248.5). Donors and subjects were age-matched as close as possible for each trial (mean difference=37.1 d, SE=3.7 d).

All hamsters used in this study were sexually naïve. The estrus cycle of

females was not monitored and thus knowledge of their estrus state was unknown. After this study was complete, many individuals were used to make breeding pairs for another experiment, serving as a test for reproductive competency. Forty-seven subjects (N = 47/60 subjects, 78.3%), the scent donors for 42 same-sex trials (N = 42/60 subjects, 70%), and the scent donors for 47 opposite-sex trials (N = 47/60 subjects, 78.3%) successfully reproduced or were observed engaging in sex, while the reproductive competency for the others remains unknown.

All trials were conducted within the colony room under red ceiling lights within four hours of the onset of darkness, a period when dwarf hamsters are most active. For each trial, a subject's home cage was placed underneath a tripod-mounted video camera and the lid was removed. The subject's wood gnawing block was repositioned a few centimeters from the walls of the end of the cage where the subject had constructed its nest in a corner. All of the subject's cotton nest material was removed and the subject was given about one minute to acclimate before starting the trial. Once the subject had moved the end of the cage where its nest was located, two grams of stimulus cotton compressed into roughly the volume of a ping-pong ball was placed on the opposite end of the cage with a gloved hand. After 10 minutes, the stimulus cotton was removed and discarded. Three clean cotton balls were provided, the lid was replaced, and the home cage was returned to its rack. Gloved hands were washed between trials. Repeated trials for each subject were separated by 5-20 d and cages were cleaned between trails. The order of trials for each subject and the order of trials within test days and across test days were randomized while balancing for the

subject-sex-stimulus combination of trials.

Behavioral scoring

Trials were video recorded with either a Samsung (Seoul, South Korea) HMX-M20 (30 frames/second; N = 16 trials) or Samsung HMX-W300 (60 frames/second; N = 164 trials) video camera set at either 1920x1080 pixel (N = 171 trials) or 1280x720 pixel resolution (N = 9 trials). The subject-sex-stimulus combination of trials was roughly balanced across these video conditions. Videos were reviewed in two Phases by an observer blind to subject sex and stimulus type. In Phase I, VLC Player (VideoLAN.org) was used to score the duration in seconds of investigation of the stimulus cotton and the duration of grooming any body part, as well as, to evaluate nest building behavior and to note potential trials that contained scent-marking. In Phase II, QuickTime Player (Apple, Inc., Cupertino, CA) was used to score the duration of grooming each body part frame by frame (converted to seconds using the video frame rate) and to carefully evaluate potential instances of scent-marking.

The duration of investigation started when the subject's nose first came into contact with the cotton stimulus and ended when the subject walked away or turned away for more than one second. The duration of investigation was not measured for the entire trial because many subjects started to construct a nest with the stimulus cotton during the trial. Stuffing the cotton into cheek pouches, or picking it up with their mouth to carry (usually back to the depression in the litter where their nest was) were also behaviors that ended the duration of investigation, even though the subject's

nose remained in contact with the cotton.

Grooming was defined as any behavior where the mouth or forepaws was rhythmically rubbed over another body part and did not include scratching behavior. In Phase I, videos were scored for the duration of grooming any body part. In Phase II, the video scorer jumped to points in the videos where a grooming bout occurred and then rescored it frame by frame for the duration of grooming separate body parts.

The identity of the separate body parts that were the target of grooming were noted in Phase I and used to determine which were scored in Phase II. Whisker grooming was when the forepaws went over the tip of the nose. Face grooming was when the forepaws went further up the muzzle beyond the nose but not over the ears and usually involved closing the eyes. Head grooming was when the forepaws went over and behind the ears. Side grooming was when the flanks and part of the back were mouthed, usually while the forearms were grasping their flank to apparently aid in twisting their head sideways for reach. Leg grooming was the mouthing of any side of the hind leg, sometimes with the leg extended. Foot grooming was mouthing of the hindpaws. Ventral grooming was the mouthing of any part of the ventral surface often distinguishable by the animal putting its head down while sitting upright, causing its flanks to stick out. While in some cases it was clear when a subject was grooming either their mid-ventral gland or their ano-genital area, their short bodies and the limited camera angles often precluded distinction, so they were scored together. The duration of grooming unknown body parts was also scored for the times when it was clear that the subject was grooming but the exact target of grooming was not visible in

the video.

The grooming of different body parts has been found to often follow a stereotypical sequence across rodent species in four "phases" (*sensu* Berridge, 1990), staring with the whiskers at the tip of the nose, progressing upward along the face, continuing over the ears, and then transitioning to the rest of the body (see Berridge, Aldridge, Houchard, & Zhuang, 2005 for visual aids). The separate body parts that were chosen to be scored as the targets of grooming for this study were determined by observing subjects, but the criteria used for whisker grooming, face grooming, and head grooming are similar to the first three phases of Berridge (1990). A tendency to progress from whiskers, to the face, and then over the ears was also observed in this study for *Phodopus sungorus*, though the grooming of other body parts did not seem as rigidly sequenced. Moreover, the amount of time that an individual spent grooming one body part varied between individuals, whether or not it occurred in a stereotypical sequence.

A very small proportion of the grooming observed took place prior to a subject's first contact with the stimulus cotton (75/3420 s, 2.2%), and the analysis of the duration of grooming any body part yielded similar results whether or not grooming prior to first contact was excluded. Furthermore, given the small size of a cage, it is very likely that a subject detected the odor of the stimulus prior to first contact, which is consistent with the very short latencies to first contact that were observed. Therefore, the analyses of all grooming behaviors presented here were based on any grooming that took place from the moment the cotton stimulus was placed at

the start of the trial.

This experiment was not designed with the scoring of scent-marking in mind and so the top-down view of the camera was not ideally suited for the task. Nevertheless, it became clear while observing the trials in person that scent-marking was occurring in several trials and so an attempt was made to score the most obvious occurrences. Trials with any potential scent-marking behavior were flagged during video review in Phase I by one observer and by in-person casual observations made while trials were being recorded by a second observer. In Phase II, these trials were carefully reviewed in their entirety for any instances of scent-marking by two observers blind to treatment. Scent-marking was when a subject appeared to press down and briefly drag its ventrum on a substrate protruding from the floor. The substrates targeted for marking where usually the wood gnawing block or the cotton stimulus, though in once instance a food pellet was marked. There were no obvious or even suspected instances of scent-marking of the cage floor, though whether or not marks were made on the floor is uncertain. Due to the camera's vantage point, it was most often unable to be determined if the subject was marking with their mid-ventral gland or ano-genital area, so both were scored as scent-marking. Due to the presence of litter in the cage, urination and defecation were not able to be observed and thus not scored as a type of scent-marking. When scent-marking, the subject often slowly climbed over the cotton stimulus or wood block, but it was often ambiguous as to whether the body was being pressed down to leave a mark. Due to this ambiguity, the counts of scent-marking events were not scored. Instead, trials were dichotomously

scored as having any scent-marking versus having no scent-marking. Fortunately, there were usually several scent-marking events that were scored with high confidence in a single trial or several lower confidence events in addition to one high confidence event.

Statistical Analysis

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, U.S.A.). Statistical significance was set at $\alpha = 0.05$. Unless otherwise specified, the sample size for analyses was $N = 180$ trials ($N = 30$ subjects for each of the six subject-sex-stimulus categories).

The latency to first contact with the cotton stimulus was analyzed using a cox proportional hazards model with the "exact" method to handle ties (PROC PHREG, SAS). To control for repeated measures, a robust sandwich estimate was used to correct the covariance matrix for clustering. The model included subject sex (male or female), stimulus type (clean, same-sex, or opposite-sex), and their interaction.

Durations of time responses were analyzed using factorial repeated measures analysis of variance (rmANOVA) models with compound symmetry covariance structures to control for repeated measures (PROC MIXED, SAS). Unless otherwise stated, the model included subject sex (male or female), stimulus type (clean, same-sex, or opposite-sex), and their interaction. *F* statistics for Type III tests of fixed effects were calculated using the "KenwardRoger2" denominator degrees of freedom option. Post-hoc pairwise comparisons were calculated using the difference of LS-

means method. The “Tukey” adjustment option was used to reduce family-wise error rate by producing *P* values adjusted for multiple comparisons and the “adjdfe=row” sub-option was used to account for the specified denominator degrees of freedom option when adjusting *P* values. Assumptions of the rmANOVA were evaluated visually (PROC UNIVARIATE), and when they were not met, response variables were log transformed. Response variables that contained any zero values had a constant (one) added to every response before log transformation. Means are presented as back transformed least-squares means (LS-means) with 95% confidence intervals.

Binary responses were analyzed using generalized estimating equations (GEE) logistic regression models with subject as a cluster variable and an exchangeable correlation structure within subjects to control for repeated measures (PROC GENMOD, SAS). Type III tests for significance of fixed effects were calculated using the default score statistics for GEE. Estimate LS-means are reported on the inverse linked scale as probabilities 95% confidence intervals. Post-hoc pairwise comparisons were calculated using the difference of LS-means method. The “Tukey” adjustment option was used to reduce family-wise error rate by producing *P* values adjusted for multiple comparisons.

Correlations between duration of investigation and durations of different grooming behaviors were analyzed using two-tailed Spearman’s rank-order correlations due to non-normality of the data. Similar results were obtained from Pearson’s correlation using transformed data, though normal distributions could not be obtained for all variables, so the results of Spearman’s correlations of non-transformed

data are reported.

Ethical Note

The methods used were approved by Cornell University's Institutional Animal Care and Use Committee (Protocol #1993-0120). At the end of the study, animals remained in the colony for use in other studies.

RESULTS

Latency to First Contact

Subjects were mostly quick to make first contact with the cotton stimulus at the start of a trial. Across all trials (N = 180), the latency to first contact ranged from 1-207 s (media$N$ = 13 s, interquartile range=2-207 s). First contact was made within three seconds of providing the stimulus in 31.7% (38/180) of trials and within one minute in 95% (171/180) of trials. In every trial, the subject eventually made contact with the stimulus. Neither stimulus, subject sex, nor their interaction had a significant effect on the latency to make first contact with the cotton stimulus (Cox Proportional Hazards: stimulus: $\chi^2_2 = 0.07$, $P = 0.966$; sex: $\chi^2_1 = 1.16$, $P = 0.281$; interaction: $\chi^2_2 = 0.11$, $P = 0.948$). Results were similar when tests were run excluding the trial with the highest latency, a possible outlier.

Duration of Investigation

The duration of investigation of the stimulus upon first encounter was log

transformed to meet the assumptions of the rmANOVA. Stimulus type and subject sex had significant effects on the model, while their interaction did not (rmANOVA: stimulus: $F_{2,116} = 14.62$, $P < 0.0001$; sex: $F_{1,58} = 6.90$, $P = 0.011$; interaction: $F_{2,116} = 1.47$, $P = 0.235$). Means for each sex by stimulus category and the significance of all pairwise differences are presented in Figure 2.1. The mean time spent investigating cotton scented with opposite-sex odor (24.36 s [18.78-31.59]) was significantly greater than the mean times spent investigating cotton scented with same-sex odor (10.74 s [8.28-13.93]; $t_{116} = 4.75$, $P < 0.0001$; Figure 2.1) and unscented clean cotton (11.01 s [8.49-14.28]; LS-means: $t_{116} = 4.75$, $P < 0.0001$; Figure 2.1). The difference between means of cotton scented with same-sex odor and unscented clean cotton was not significant (LS-means: $t_{116} = 0.14$, $P = 0.989$; Figure 2.1). Females, on average, spent a significantly longer time investigating stimuli than males (females: 17.85 s [13.98-22.79]; males: 11.34 s [8.88-14.48]; LS-means: $t_{58}=2.63$, $P = 0.011$; Figure 2.1).

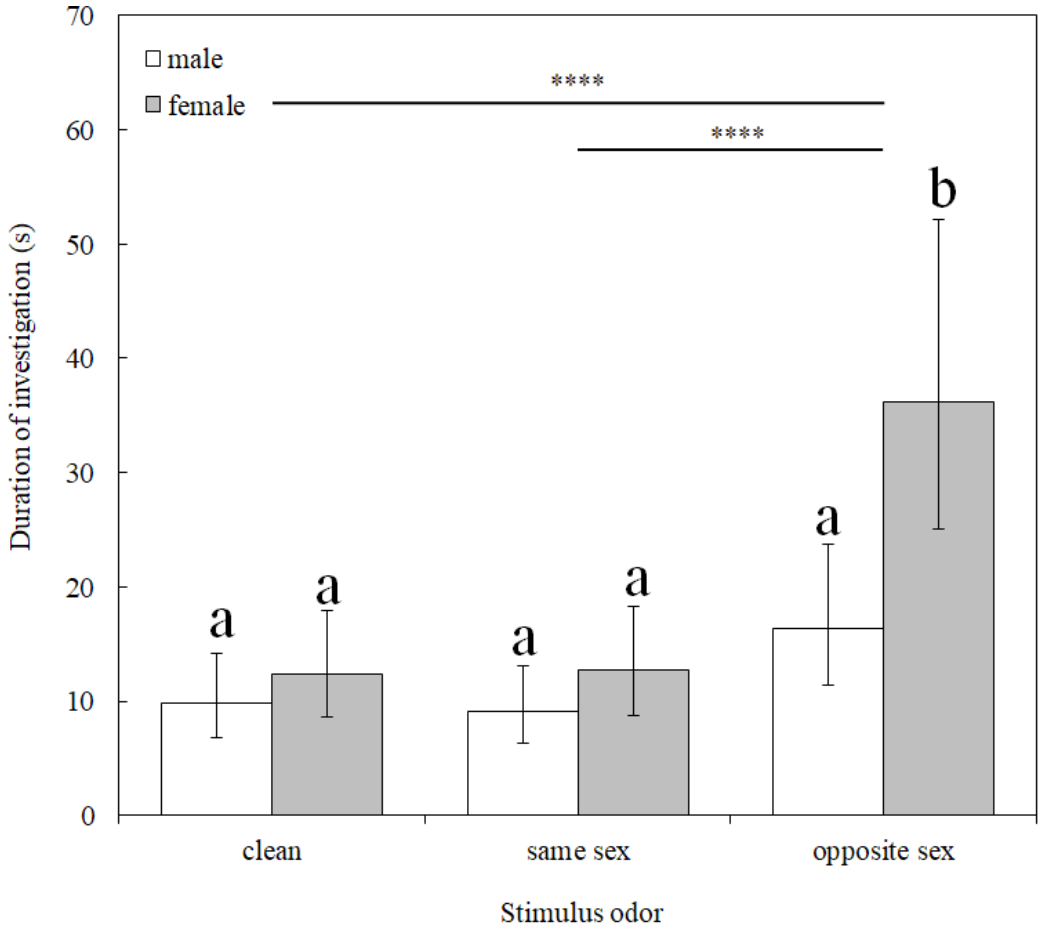

Figure 2.1. Duration of investigation upon first encounter by male and female *Phodopus sungorus* hamsters of a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent back-transformed LS-means with 95% confidence intervals. Significance tests were performed on differences of LS-means and adjusted for multiple comparisons. Bars with different letters are significantly different from one another. Horizontal lines indicate significant differences between stimulus odor categories pooled for sex and "****" indicates $P < 0.0001$. Sexes were also significantly different pooled across stimulus odor categories with females>males ($P = 0.0110$; see text).

Since cotton was a familiar nest-building material to subjects, I explored the possibility that duration of contact with the cotton stimulus may have reflected nest-building behavior and not investigation behavior. Subjects used the cotton stimulus to construct a nest in 61.1% of trials (N = 110/180). The probability of building a nest during a trial was significantly affected by stimulus type, but not subject sex, nor their interaction (logistic regression: stimulus: $\chi^2_2 = 15.5$, $P = 0.0004$; sex: $\chi^2_1 = 0.94$, $P = 0.333$; interaction: $\chi^2_2 = 4.6$, $P = 0.100$). The mean probability that a nest was built with unscented clean cotton (0.78 [0.66-0.87]) was significantly greater than the mean probabilities that a nest was built with cotton scented with same-sex odor (0.61 [0.47-0.73]; LS-means: $Z = 2.43$, $P = 0.040$; Figure 2.2) and cotton scented with opposite-sex odor (0.45 [0.33-0.58]; LS-means: $Z = 4.14$, $P = 0.0001$; Figure 2.2). The difference between means of cotton scented with same-sex odor and cotton scented with opposite-sex odor was not significant (LS-means: $Z = 2.10$, $P = 0.090$; Figure 2.2). The overall pattern of nest building across stimulus type is notably different from that of duration of investigation (Figures 2.1 & 2.2). Moreover, rerunning the rmANOVA of duration of investigation with nest building and all interactions in the model yielded similar results to the original model. Stimulus type and subject sex remained significant while nest building and all interactions were not significant (Table 2.1). Additionally, the mean duration of investigation was higher in trials without nest building (16.54 s [12.51-21.88]) than in trials with nest building (12.35 s [9.94-15.35]), though this trend was not significant (LS-means: $t_{162} = 1.70$, $P = 0.0912$).

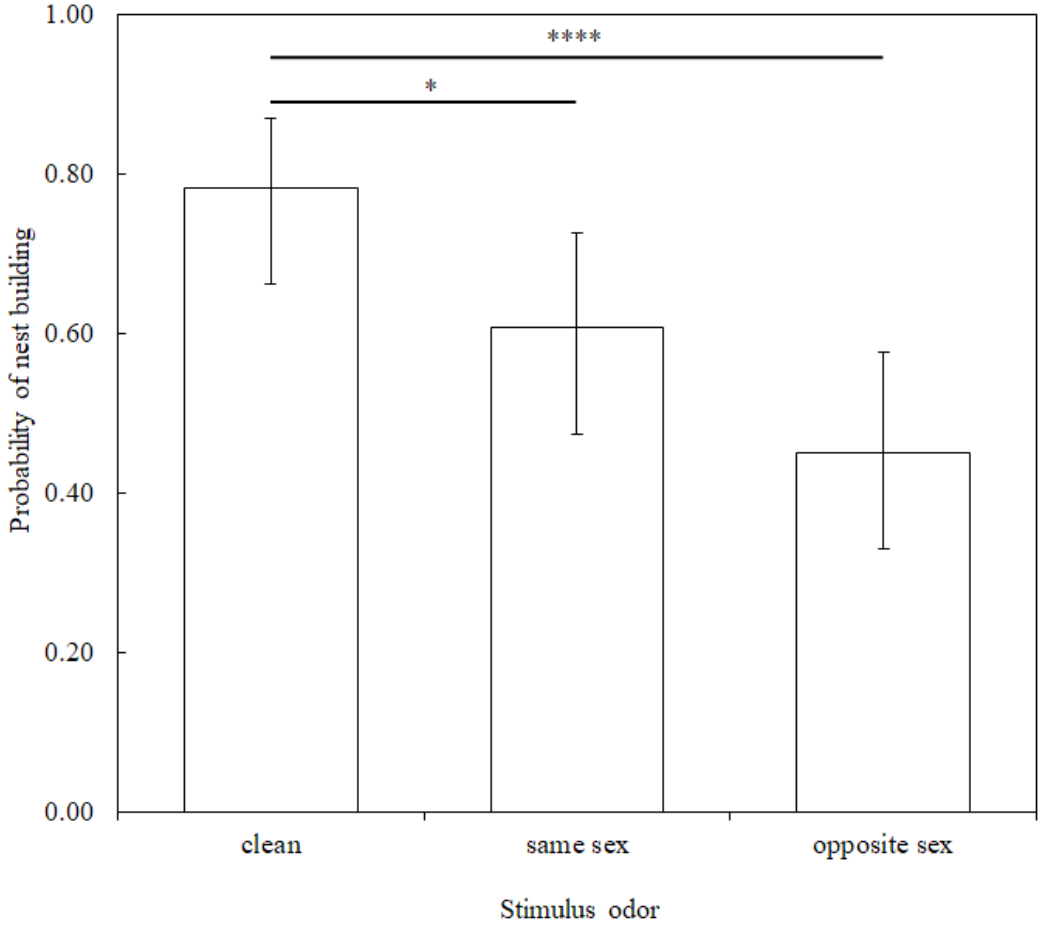

Figure 2.2. Probability of male and female *Phodopus sungorus* hamsters building a nest with a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Horizontal lines indicate significant differences of LS-means between stimulus odor categories pooled for sex and adjusted for multiple comparisons ("****" indicates $P < 0.0001$, "*" indicates $P < 0.05$).

Table 2.1. Results of a rmANOVA analyzing the duration of investigation upon first encounter by male and female *Phodopus sungorus* hamsters of a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. The model includes the effects of subject sex, stimulus odor type, whether or not nest building occurred, their three-way interaction, and all two-way interactions. The effects of subject sex and stimulus type remained significant despite the inclusion of nest building and its interactions in the model. There was also an interesting trend in the pattern of nest building (see text). The "*" symbol emphasizes statistically significant *P* values.

Effect	*F*	*df*	*P*
subject sex	4.68	1,69.5	*0.0339
stimulus	12.35	2,122	*<0.0001
nest building	2.89	1,162	0.0912
subject sex*stimulus	2.26	2,122	0.1084
subject sex*nest building	3.22	1,162	0.0746
stimulus*nest building	0.49	2,150	0.6116
subject sex*stimulus*nest building	0.18	2,150	0.8320

Grooming

Grooming of any body part occurred in a large proportion of trials (N = 166/180, 92.2%) and a total of 57 min of grooming was observed over all 1800 min of observations (3.2%). All subjects except one groomed in at least one of their three trials (N = 59/60, 98.3%), and most groomed in all three (N = 49/60, 81.7%).

The duration of grooming any body part with the mouth or forepaws in response to a stimulus odor was log transformed (log[y+1]) to meet the assumptions of the rmANOVA. Stimulus type and its interaction with subject sex had significant effects on the model, while subject sex alone did not (rmANOVA: stimulus: $F_{2,116}$ = 20.48, $P < 0.0001$; sex: $F_{1,58} = 0.22$, $P = 0.640$; interaction: $F_{2,116} = 4.64$, $P = 0.0115$). Means for each sex by stimulus category and significance of pairwise differences are presented in Figure 2.3. The mean duration of grooming by females in response to opposite-sex odor stimuli was significantly greater than grooming in response to either same-sex stimuli (LS-means: $t_{116} = 5.57$, $P < 0.0001$) or unscented controls (LS-means: $t_{116} = 5.89$, $P < 0.0001$; Figure 2.3). Note that while no pairwise difference between stimulus odor types within males was statistically significant after controlling for multiple comparisons (all $P > 0.1329$), the pattern across stimulus odor types was similar between sexes, with grooming being highest in response to opposite-sex odor (Figure 2.3).

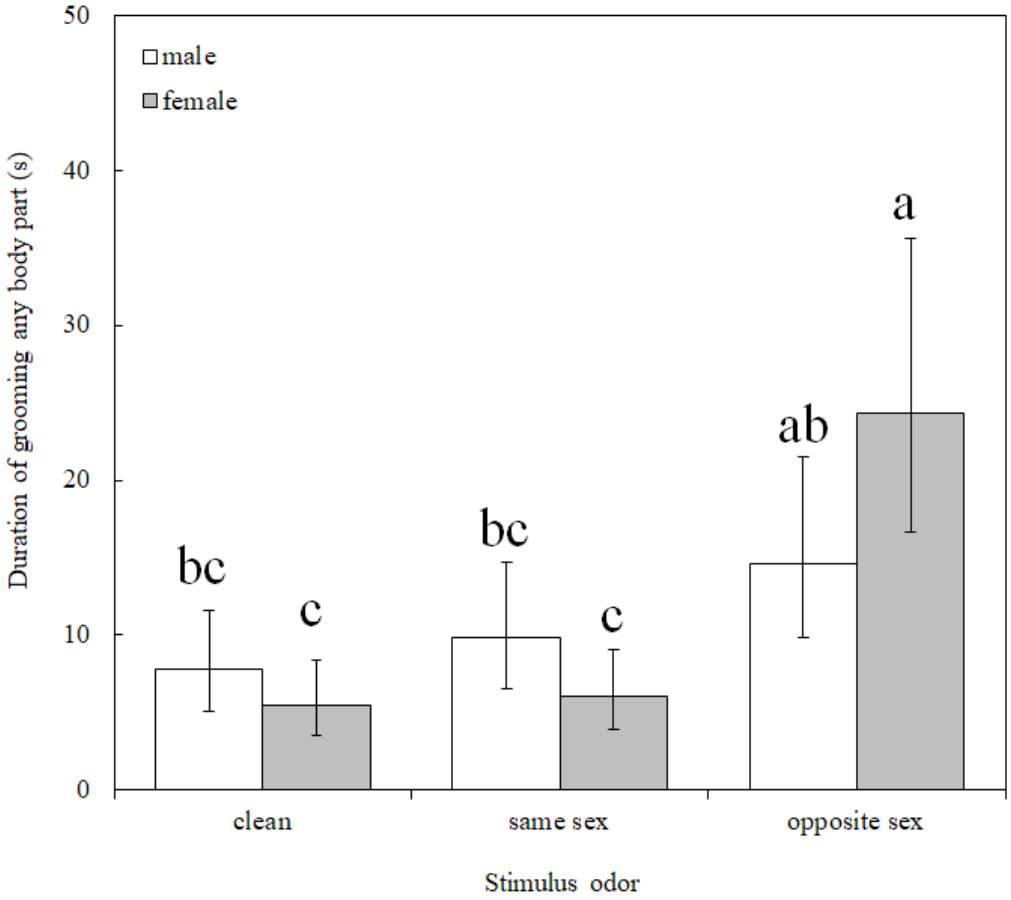

Figure 2.3. Duration of grooming of any body part with the mouth or forepaws by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent back-transformed LS-means with 95% confidence intervals. Bars with different letters are significantly different from one another after adjusting for multiple comparisons.

Since nest building with the cotton stimulus involves a moderate amount of body contact with the cotton, I explored the possibility that the duration of grooming any body part was influenced by nest building behavior. Rerunning the rmANOVA after adding nest building and all interactions in the model yielded similar results to the original model. Stimulus type and the interaction of stimulus type and subject sex remained significant (Table 2.2). Additionally, nest building had a significant effect on the model (Table 2.2). Differences of LS-means tests revealed the mean duration of grooming was significantly higher in trials without nest building (14.91 s [11.12-19.90]) than in trials with nest building (7.39 s [5.76-9.42]; LS-means: $t_{167} = 3.94$, $P = 0.0001$).

Table 2.2. Results of a rmANOVA analyzing the duration of grooming any body part by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. The model includes the effects of subject sex, stimulus odor type, whether or not nest building occurred, their three-way interaction, and all two-way interactions. The effects of stimulus type and the interaction of stimulus type and subject sex remained significant while the effect of subject sex remained nonsignificant despite the inclusion of nest building and its interactions in the model. The "*" symbol emphasizes statistically significant *P* values.

Effect	*F*	*df*	*P*
subject sex	0.06	1,66.5	0.8026
stimulus	14.72	2,119	*<0.0001
nest building	4.51	2,119	*0.0130
subject sex*stimulus	15.49	1,167	*0.0001
subject sex*nest building	2.43	1,167	0.1206
stimulus*nest building	1.06	2,141	0.3490
subject sex*stimulus*nest building	1.20	2,141	0.3052

The duration of whisker grooming was the only grooming response for a separate body part that was able to meet the assumptions of the rmANOVA after log transformation (log[y+1]). Stimulus type and its interaction with subject sex had significant effects on the model, while subject sex alone did not (rmANOVA: stimulus: $F_{2,116} = 13.03$, $P < 0.0001$; sex: $F_{1,58} = 0.58$, $P = 0.449$; interaction: $F_{2,116} = 3.47$, $P = 0.0342$). Means for each sex by stimulus category and significance of pairwise differences are presented in Figure 2.4. The mean duration of whisker grooming by females in response to opposite-sex odor stimuli was significantly greater than whisker grooming in response to either same-sex stimuli (LS-means: $t_{116} = 4.39$, $P < 0.0004$) or unscented controls (LS-means: $t_{116} = 5.01$, $P < 0.0001$; Figure 2.4). Note that while no pairwise difference between stimulus odor types within males was statistically significant after controlling for multiple comparisons, the pattern across stimulus odor types was similar between sexes, with grooming being highest in response to opposite-sex odor (Figure 2.4). Whisker grooming was also analyzed as a binary response for comparison with grooming of other body parts, but due in part to a large proportion of trials in which whisker grooming was observed ($N = 166/180$, 92.2%), no factors were significant in the model (Table 2.3).

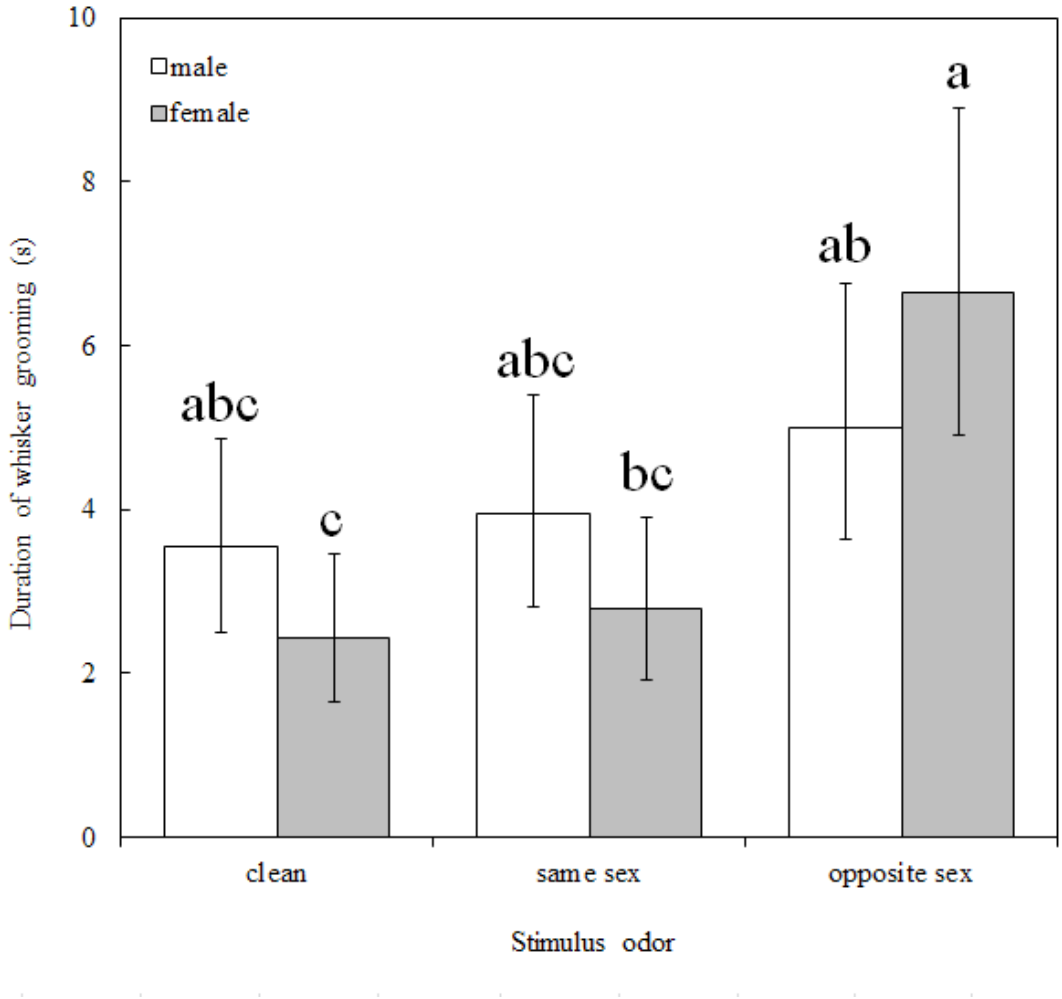

Figure 2.4. Duration of whisker grooming by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent back-transformed LS-means with 95% confidence intervals. Bars with different letters are significantly different from one another after adjusting for multiple comparisons.

Table 2.3. Results of a repeated measures GEE logistic regression analyzing the probability of grooming separate body parts by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. For each body part, except ventral, the model includes the effects of subject sex, stimulus odor type, and their interaction. Due to zero instances of grooming by females in clean and same-sex trials, subject sex and its interaction with stimulus were removed from the model (see text). The "*" symbol emphasizes statistically significant *P* values and the "†" symbol emphasizes marginally nonsignificant *P* values. See text for descriptions of grooming targets.

	subject sex			stimulus			interaction		
Grooming Target	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
whiskers	1.22	1	0.2689	1.30	2	0.5229	0.68	2	0.7102
face	5.26	1	*0.0218	1.07	2	0.5847	1.60	2	0.4504
head	0.83	1	0.3635	6.02	2	*0.0494	0.11	2	0.9479
side	5.38	1	*0.0204	5.79	2	†0.0554	0.04	2	0.9789
leg	0.02	1	0.8973	5.97	2	†0.0505	1.67	2	0.4337
foot	0.58	1	0.4475	4.29	2	0.1172	2.79	2	0.2477
ventral				17.49	2	*0.0002			
unknown	6.57	1	*0.0104	17.53	2	*0.0002	7.00	2	*0.0301

The durations of grooming each body part were converted into binary responses and analyzed with GEE logistic regression models. Table 2.3 provides a summary of the statistical results for each model. The mean probability of grooming each body part in each of the three stimulus conditions is summarized in Figure 2.5; for all body parts, the probability of grooming tended to be greater in trials with cotton scented with conspecific odor than in trials with unscented clean cotton, and for all but one body part, the probability of grooming in response to opposite-sex conspecific odor tended to be highest.

Stimulus type had a significant effect on the probability of grooming the head, though pairwise differences of LS-means were not significant after adjusting for

multiple comparisons (LS-means: clean-opposite: $Z = 2.25$, $P = 0.063$; clean-same: $Z = 0.20$, $P = 0.978$; same-opposite: $Z = 1.97$, $P = 0.119$; Table 2.3 & Figure 2.5). Stimulus type had a marginally nonsignificant effect on the probability of grooming the side, though tests of differences of LS-means found that the mean probability of grooming the side in response to cotton scented with opposite-sex odor was significantly greater than in response to unscented clean cotton (LS-means: $Z = 2.38$, $P = 0.045$; Table 2.3 & Figure 2.5). The probability of grooming the side in response to cotton scented with same-sex odor was neither significantly different from either cotton scented with opposite-sex odor nor unscented cotton (LS-means: clean-same: $Z = 0.83$, $P = 0.686$; same-opposite: $Z = 1.87$, $P = 0.149$; Figure 2.5). Stimulus type had a marginally nonsignificant effect on the probability of grooming the leg and tests of differences of LS-means did not find any significant differences between stimulus types (LS-means: clean-opposite: $Z = 2.21$, $P = 0.069$; clean-same: $Z = 0.68$, $P = 0.774$; same-opposite: $Z = 1.86$, $P = 0.149$; Table 2.3 & Figure 2.5). Subject sex had a significant effect on the probability of grooming the face and, separately, the side (Table 2.3). Males, on average, had a higher probability than females of grooming the face (males: 0.86 [0.77-0.92]; females: 0.68 [0.55-0.79]; LS-means: $Z = 2.51$, $P = 0.0120$) and grooming the side (males: 0.34 [0.23-0.47]; females: 0.15 [0.08-0.25]; LS-means: $Z = 2.44$, $P = 0.0147$).

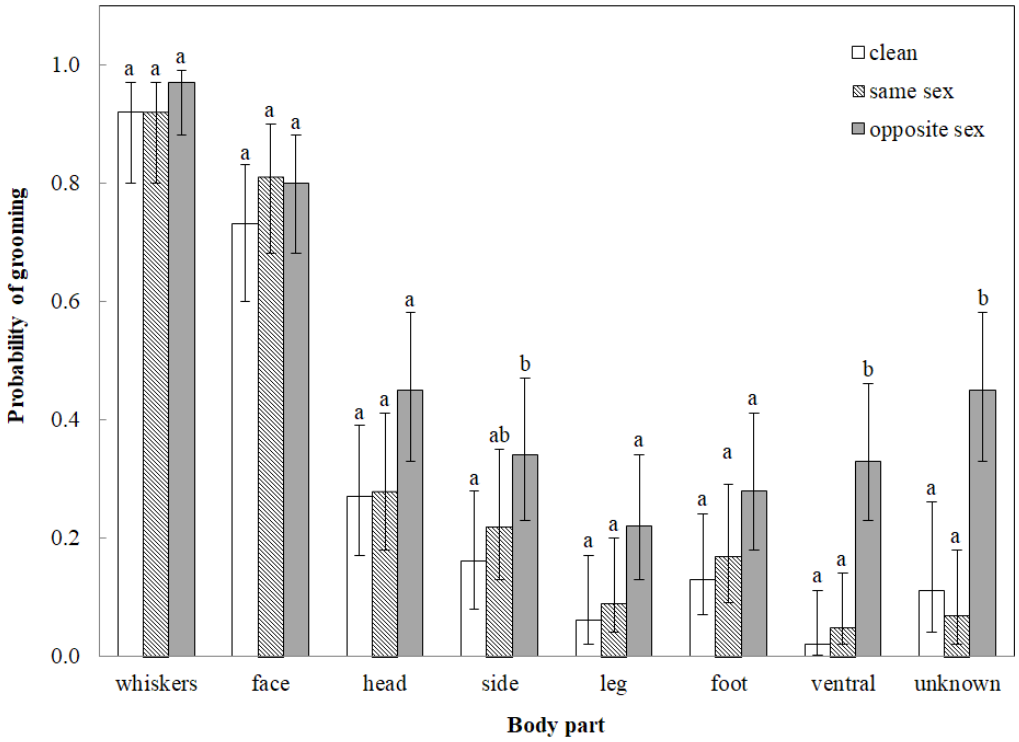

Figure 2.5. Probability of grooming separate body parts by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. The GEE logistic regression models used to generate these values contained subject sex, stimulus type, and their interaction, except in the case of ventral grooming which had to exclude subject sex and the interaction due to zero instances of grooming by females in clean and same-sex trials. Separately for each body part, bars with different letters are significantly different from one another after adjusting for multiple comparisons. See text for descriptions of grooming targets.

Due to the absence of any grooming of the ventral surface in two stimulus-by-sex categories, subject sex and its interaction with stimulus could not be included in the logistic regression model. No female subjects groomed their ventral surface in either trials with an unscented clean cotton stimulus or trials with a cotton stimulus scented with same-sex odor, while half of females (N = 15/30, 50%) groomed in trials with a cotton stimulus scented with opposite-sex odor. Nine different male subjects groomed their ventral surface in a single trial, with the highest number of trials in the opposite-sex stimulus category (clean: N = 1/30, 3.3%; same-sex: N = 3/30, 10%; opposite-sex: N = 5/30, 16.7%). A GEE logistic regression model was run with only stimulus as a main effect, effectively pooling the sexes, and its effect was significant (Table 2.3). Tests for differences of LS-means found that the mean probability of grooming the ventral surface in trials with cotton scented by opposite-sex odor was significantly higher than the mean probabilities for trials with same-sex cotton (LS-means: Z = 3.25, P = 0.003) or unscented cotton (LS-means: Z = 3.17, P = 0.004; Figure 2.5), while the mean probability for same-sex cotton was not significantly different from unscented cotton (LS-means: Z = 0.96, P = 0.605; Figure 2.5). Rerunning the GEE logistic regression model using only male subjects and with stimulus type as the only independent variable, stimulus was found to not have a significant effect (logistic regression: χ^2_2 = 3.13, P = 0.209) and no difference of LS-means was found to be significant either (LS-means: clean-opposite: Z = 1.51, P = 0.287; clean-same: Z = 0.96, P = 0.601; same-opposite: Z = 0.70, P = 0.762).

The probability of grooming an unknown body part was significantly affected

by the interaction between stimulus type and subject sex (Table 2.3). The patterns of pairwise differences were complicated and are shown in Figure 2.6. For females, the pattern tended to be that the probability of grooming an unknown body part was highest for the opposite-sex stimulus and about equally lower for a same-sex stimulus and an unscented stimulus. For males, the pattern tended to be that the probability of grooming an unknown body part was highest for the opposite-sex stimulus, but second highest for the same-sex stimulus. The mean probabilities for each stimulus type pooled for sex is presented in Figure 2.5.

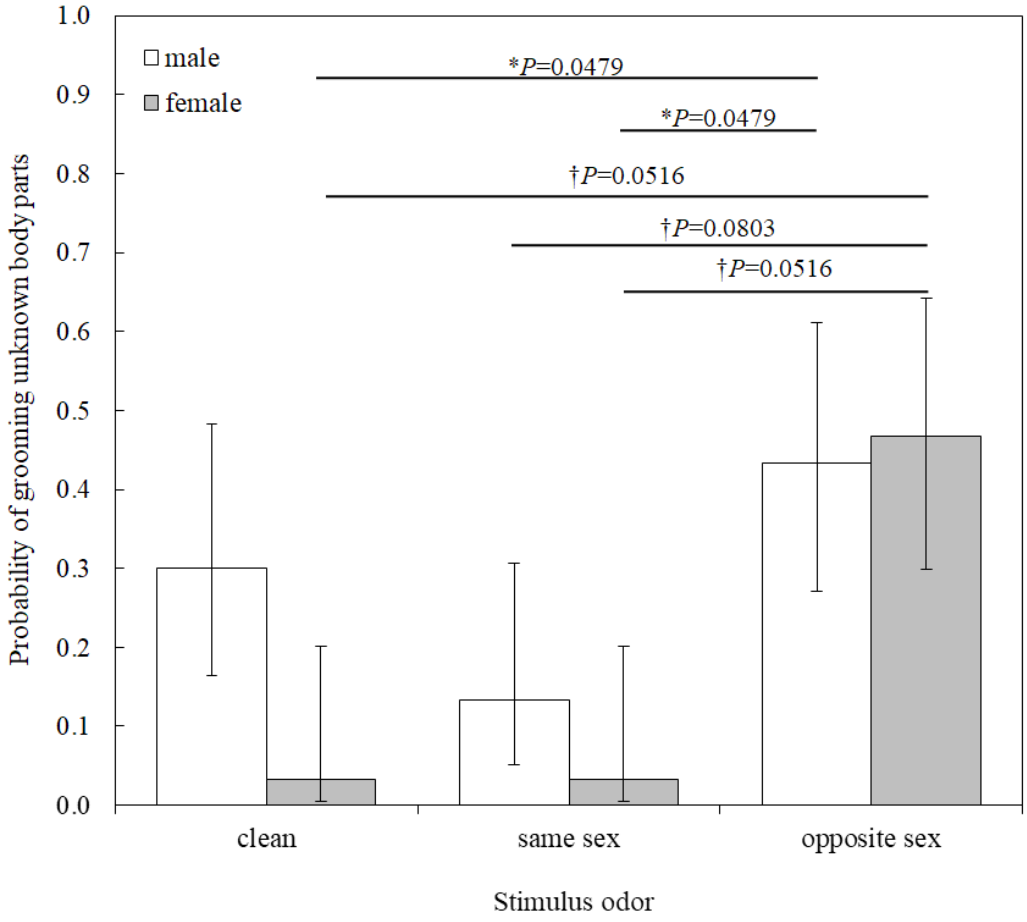

Figure 2.6. Probability of grooming unknown (due to an obfuscated view) body parts by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus that was unscented (clean) or scented with odor from a same-sex or opposite-sex conspecific. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Horizontal bars with *P* values indicate significant (*) or marginally nonsignificant (†) differences of LS-means.

Correlations between investigation and grooming

To see if self-grooming possibly reflects a subject's interest in the stimulus odor, I examined the linear relationship between duration of self-grooming and the duration of investigation upon first contact. Since subjects were tested once for each stimulus type, correlations were analyzed separately for each stimulus type to avoid pseudo-replication. For trials in which the stimulus odor was the opposite-sex, the duration of investigation significantly and positively correlated with the duration of grooming any body part, whisker grooming, ventral grooming, and grooming of unknown body parts (Table 2.4). There was also a marginally nonsignificant positive correlation with head grooming (Table 2.4). Grooming did not significantly correlate with investigation for any body part when the stimulus was unscented clean cotton or cotton scented by same-sex odor (Table 2.4).

Table 2.4. Results of Spearman rank correlation analyses between the duration of investigation upon first contact with a cotton stimulus and the duration of grooming different body parts by *Phodopus sungorus* hamsters. For each analysis, $N = 60$ subjects (30 males and 30 females). Each subject was tested once with each of three stimulus types: unscented (clean) cotton, cotton scented with same-sex odor, and cotton scented with opposite-sex odor. *P* values are emphasized as statistically significant (*) or marginally non-significant (†).See text for descriptions of grooming targets.

	clean		same-sex		opposite-sex	
	r_s	*P*	r_s	*P*	r_s	*P*
Grooming Target						
any body part	0.001	0.996	-0.130	0.322	0.270	*0.037
whiskers	0.053	0.689	-0.079	0.547	0.311	*0.016
face	-0.049	0.711	-0.209	0.109	0.138	0.292
head	-0.096	0.464	-0.077	0.561	0.234	†0.072
side	-0.002	0.991	-0.080	0.542	-0.016	0.902
leg	-0.023	0.861	0.119	0.367	0.061	0.644
foot	-0.028	0.830	0.154	0.239	0.060	0.651
ventral	0.011	0.932	0.112	0.395	0.311	*0.016
unknown	0.194	0.138	0.045	0.734	0.346	*0.007

To see if these relationships differed between sexes, Spearman's rank correlations were run separately for each sex by stimulus combination (N = 30 per analysis). For both males and females, there were no statistically significant correlations when the stimulus was unscented clean cotton (data not shown), though the positive correlation between duration of investigation and grooming unknown body parts was on the cusp of statistical significance for male subjects (Spearman's rank correlation: $r_s = 0.361$, $N = 30$, $P = 0.0500$). For females, no correlation was significant when the stimulus was cotton scented by same-sex odor ($P > 0.233$), and for males only foot grooming was significant (Spearman's rank correlation: $r_s = 0.380$, $N = 30$, $P = 0.039$). When the stimulus was cotton scented with opposite-sex odor, no correlation was significant for female subjects ($P > 0.231$), while what was significant for males was nearly the same as what was significant when both sexes were pooled for analysis (Table 2.5).

Table 2.5. Results of Spearman rank correlation analyses between the duration of investigation upon first contact with a cotton stimulus scented with opposite-sex odor and the duration of grooming different body parts by male and female *Phodopus sungorus* hamsters. For each analysis, $N = 30$ subjects. *P* values are emphasized as statistically significant (*) or marginally non-significant (†). See text for descriptions of grooming targets.

	males		females	
	r_s	*P*	r_s	*P*
Grooming Target				
any body part	0.361	†0.050	-0.076	0.690
whiskers	0.370	*0.044	0.179	0.345
face	0.134	0.480	0.163	0.389
head	0.353	†0.056	0.099	0.601
side	0.081	0.670	0.005	0.979
leg	0.210	0.266	0.003	0.989
foot	0.098	0.607	-0.226	0.231
ventral	0.406	*0.026	-0.061	0.748
unknown	0.539	*0.002	0.028	0.883

Scent-marking

Scent-marking was confidently observed for 20 different subjects (*N* = 20/60 subjects, 33.3%), 19 of which marked in only one of their three trials and one of which marked in two (*N* = 21/180 trials, 11.7%). Table 2.6 provides a breakdown of the number of trials in which marking occurred according to subject sex, stimulus type, and the target of marking. For females, the cotton stimulus was a target of scent-marking in more trials than was the home cage's wood gnawing block (cotton: *N* = 9/14, 64%; block: *N* = 5/14, 35.7%; Table 2.6). All trials with any marking were pooled for analysis, though results were similar when excluding trials with only block marking (data not shown). For both males and females, marking was not observed in any of the trials with an unscented clean cotton stimulus (Table 2.6), so that level of the stimulus variable was excluded from the GEE Logistic regression analysis in order for it to run successfully.

Table 2.6. Counts of 10-minute trials in which male and female *Phodopus sungorus* hamsters confidently scent marked in response to either a cotton stimulus scented with same-sex odor, opposite sex odor, or unscented clean cotton. Counts are separated into trials in which only the cotton stimulus itself was the target of marking, only the home cage's wood gnawing block was the target, both were the target, and either were the target.

	males			females		
Marking target	clean	same-sex	opposite-sex	clean	same-sex	opposite-sex
cotton stimulus	0	0	1	0	1	7
wood block	0	1	0	0	0	4
both	0	1	5	0	0	1
either	0	2	6	0	1	12

Stimulus type had a significant effect on the probability of scent-marking during a trial, while subject sex and the interaction between stimulus type and subject sex did not (logistic regression: stimulus: $\chi^2_1 = 9.93$, $P = 0.002$; sex: $\chi^2_1 = 0.03$, $P = 0.859$; interaction: $\chi^2_1 = 1.35$, $P = 0.245$). The mean probability of scent-marking during a trial with a cotton stimulus scented with opposite-sex odor (0.29 [0.19-0.42]) was significantly greater than the mean probability of marking in a trial with a cotton stimulus scented with same-sex odor (0.05 [0.01-0.14]; LS-means: $Z = 3.06$, $P = 0.002$; Figure 2.7). Note that for both the same-sex and opposite-sex stimulus, the 95% confidence intervals of the mean probabilities of marking do not include zero, which is how many trials involved marking in the clean stimulus condition.

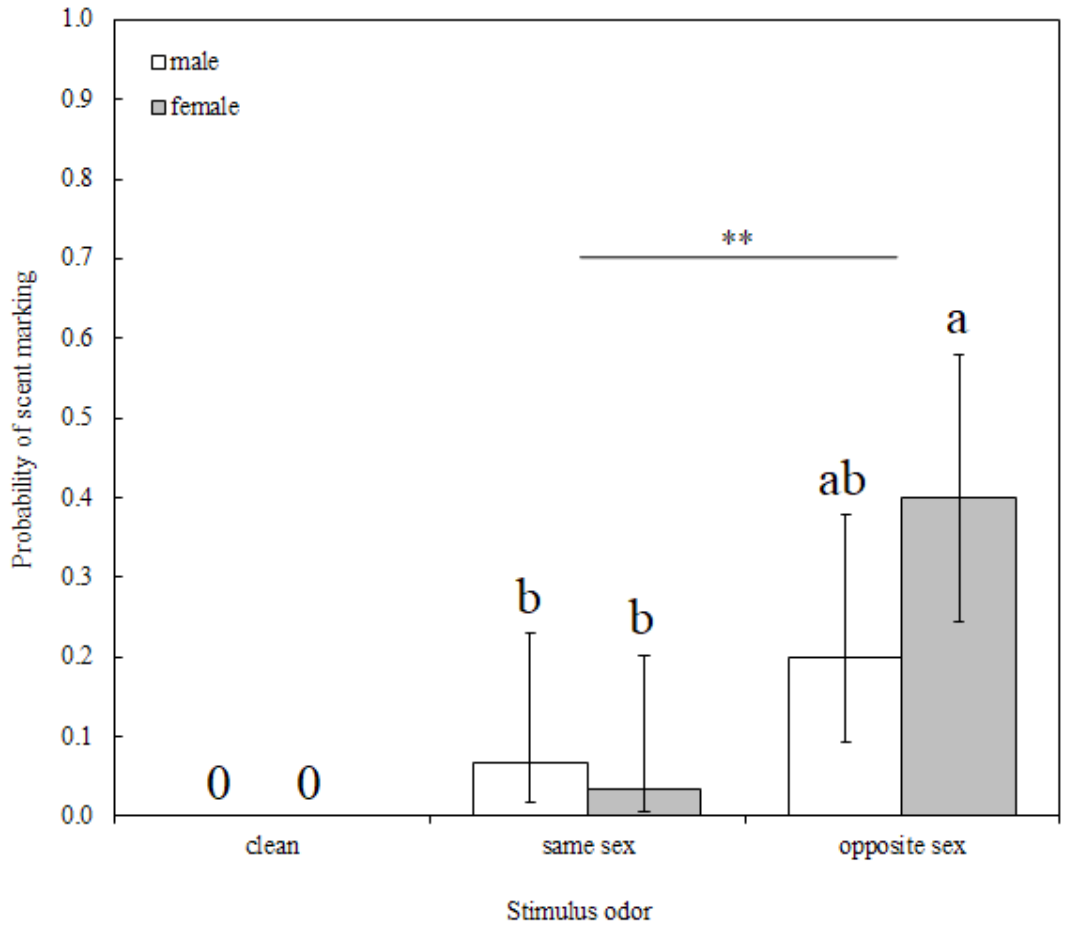

Figure 2.7. Probability of scent-marking by male and female *Phodopus sungorus* hamsters in response to a cotton stimulus scented with same-sex odor, opposite sex odor, or unscented (clean) cotton. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Bars with different letters are significantly different from one another after adjusting for multiple comparisons. The horizontal line indicates a significant difference of LS-means between same-sex and opposite-sex stimulus odor categories pooled for sex and adjusted for multiple comparisons ("**" indicates $P = 0.002$). In no trials for the clean stimulus category did males or females scent mark (represented by "0"); notice that the 95% confidence intervals for other categories do not include zero.

Since scent-marking involves dragging the body over a target substrate, material from the substrate may rub off onto the body thereby inducing an increase in overall grooming. To see if marking influenced the duration of grooming any body part, the rmANOVA from earlier was rerun with marking and all interactions with marking in the model. Due to no subject marking in trials with unscented clean cotton, this level of the stimulus variable was excluded from analysis. The effect of marking was significant in the model (rmANOVA: $F_{1,106} = 9.59$, $P = 0.003$). The mean duration of grooming any body part was significantly higher in trials with scent-marking (30.75 s [15.61-59.67]) than without (10.16 s [7.94-12.93]; LS-means: $t_{106} = 3.10$, $P = 0.003$).

However, despite including marking and interactions with marking in the model, the effect of stimulus type remained significant while the effect of the interaction between stimulus type and subject sex became nonsignificant (rmANOVA: stimulus: $F_{1,92.4} = 4.29$, $P = 0.041$; interaction: $F_{1,92.4} = 0.24$, $P = 0.625$). The mean duration of grooming any body part was significantly higher in response to a cotton stimulus scented with opposite-sex odor (25.35 s [18.4-34.15]) than same-sex odor (12.45 s [6.33-23.67]; LS-means: $t_{92.4} = 2.04$, $P = 0.041$). All other variables in the model were not significant ($P > 0.318$).

Since the ventral surface of the body is the part most likely affected by transfer of material from the target substrate while scent-marking, I reran a GEE logistic regression from earlier to see if the probability of ventral grooming was affected by marking. Subjects were pooled for sex, as in the original model, and trials from the

unscented clean cotton level of the stimulus type variable were excluded due to the lack of marking. The effect of stimulus changed to being only marginally non-significant, while marking and their interaction was not significant (rmANOVA: stimulus: $\chi^2_1 = 2.78$, $P = 0.095$; marking: $\chi^2_1 = 0.96$, $P = 0.327$; interaction: $\chi^2_1 = 0.05$, $P = 0.818$). The difference of LS-means test, however, found that the probability of grooming the ventral surface of the body was significantly higher in trials with cotton scented like opposite-sex odor (0.40 [0.27-0.56]) than same-sex odor (0.11 [0.03-0.35]; LS-means: $Z = 2.09$, $P = 0.037$). With larger datasets, the significances of these two tests usually agree, but since this model has a reduced samples size, they do not. Nevertheless, the pattern remains the same in that the probability of ventral grooming was higher for the opposite-sex stimulus condition than both the same-sex and clean control conditions, despite the inclusion of scent-marking in the model.

As an additional check to see if scent-marking influenced ventral grooming, I looked at only those trials in which ventral grooming occurred ($N = 24$ trials; 8 males [1 clean, 3 same-sex, 5 opposite-sex] and 15 females [all opposite-sex]) to see if the mean duration of ventral grooming was different between trials with and without scent-marking. The duration of ventral grooming was log transformed to better fit the model. Marking did not have a significant effect (ANOVA: $F_{1,22} = 0.43$, $P = 0.519$). The mean duration of ventral grooming in trials with scent-marking (18.95 s [7.40-48.55]) was not significantly different from trials without scent-marking (12.22 s [4.39-33.98]; LS-means: $t_{22} = 0.66$, $P = 0.519$).

DISCUSSION

Overall, this study lends support for the hypothesis that self-grooming plays a role in olfactory communication in the dwarf hamster, *Phodopus sungorus*. The prediction that self-grooming would be higher in response to the odor of conspecifics than to unscented controls was met. Specifically, self-grooming in response to opposite-sex odor was higher than same-sex odor and unscented controls, which did not differ from each other, suggesting that a communicative function for self-grooming for both males and females is to attract a mate. Subjects showed the greatest interest, as measured by duration of investigation upon first contact, in stimulus cotton scented with opposite-sex odor and this interest positively correlated with the duration of self-grooming. Subjects also showed a greater interest in communicating with the opposite-sex through scent-marking behavior. The latency to first contact with the stimulus cotton was similar across conditions, which is not unsurprising given how fast most subjects approached the stimulus after it was placed in their home cage.

For each separate body part analyzed (whiskers, face, head, side, leg, foot, ventral, unknown), the probability of grooming tended to be greater in trials with cotton scented with conspecific-odor than trails with unscented cotton, and for all but one body part (face), the probability of self-grooming in response to opposite-sex odor tended to be the highest. The probability of whisker grooming was not significantly affected by stimulus type, but this is likely due to the fact that whisker grooming occurred in a high percentage of trials. Whisker grooming was the only body part for which analysis could be done on the duration of self-grooming, and analysis of its

duration yielded similar results to the analysis of the duration of grooming of all body parts pooled together. Aside from olfactory communication, another possible explanation for the observed pattern of whisker grooming is that when subjects were more interested in a stimulus odor, they spent more time grooming their whiskers in order to gather more information about the stimulus by improving, through cleaning, the tactile sensory input provided by whiskers.

Face grooming was the second most common form of grooming, and the probability of face grooming was also not significantly affected by stimulus type, but, unfortunately, analysis of face grooming duration was not possible without violating assumptions of the statistical model. The probability of head grooming was lower than the probability of whiskers and face grooming, and was significantly affected by stimulus type. That whisker grooming followed by face grooming and then head grooming were the most common forms of grooming is perhaps unsurprising given that self-grooming in rodents follows a stereotypical sequence, starting with the whiskers and tip of nose, proceeding up the muzzle to the face, then over the ears and head, before proceeding to the rest of the body (Berridge, 1990). Although grooming sequence was not closely analyzed for the current study, I was left with the impression that grooming whiskers, face, and then head was a common sequence. If grooming sequences are very stereotyped in *Phodopus sungorus*, then most grooming bouts should start with whisker grooming and may not necessarily progress to subsequent body parts. However, this sequence is not absolute and more caudal body parts were sometimes observed being groomed without first grooming the whiskers, nose, and

head in sequence.

Face grooming usually involves the forepaws passing over closed eyes. *Phodopus sungorus* have Harderian glands behind their eyes and grooming the face may function to squeeze secretion out of the gland that drips internally into the nares, while the whiskers, face, and head sequence may function to spread the secretion that discharges from the nares over the face and head (e.g., Payne, 1977; Shanas & Terkel, 1995; Thiessen et al., 1976). Alternatively, or additionally, grooming of the whiskers, face, and head may function to spread other communicative odors around the body, including saliva and sacculus secretion. Communicating sexual identity is an important precursor to mating. Saliva has been shown to be used by males, but not females, of *Phodopus campbelli* to distinguish between sexes (Lai, Vasilieva, & Johnston, 1996), while sacculus secretion has been shown to be used to distinguish between sexes by males of *Phodopus sungorus* and males, but not females, of *Phodopus campbelli* (Feoktistova, 1994; Lai et al., 1996). *Phodopus sungorus* males made a higher number of ano-genital scent marks in response to female sacculus secretion than male sacculus secretion, further suggesting that sacculus secretion functions to attract a mate (Feoktistova, 1994). Saliva and sacculus secretion may also communicate information about female reproductive state, as *Phodopus campbelli* males preferred these odors from female donors that were in post-partum estrus over female donors in non-estrus (Lai et al., 1996). Subject sex had a significant effect on face grooming, with males being more likely to face groom than females. This might suggest that males rely on face grooming more than females for olfactory

communication. Perhaps contrary to this possibility, *Phodopus campbelli* females did not use saliva to distinguish between sexes while males did (Lai et al., 1996). Despite finding in the current study a significant effect of stimulus on head grooming, which involves the forepaws going over the ears, male *Phodopus campbelli* did not show a preference for odor from behind the ear for either sex (Lai et al., 1996). This may hint at a species difference, but additional experimentation using ear odor is necessary.

The effect of stimulus type on side grooming was only marginally non-significant, though pairwise comparisons showed that side-grooming in response to opposite-sex odor was significantly greater than in response to unscented controls. Side grooming, as defined in this study, included grooming of the back. *Phodopus* species do not have dedicated scent glands on their sides or backs like some other rodent species do, and back fur odor was not used by males of *Phodopus campbelli* to distinguish between sexes (Lai et al., 1996). Nevertheless, *Phodopus sungorus* males were not able to be distinguished from other male individuals when their fur was cleaned with detergents, suggesting that their sides and backs may still carry communicative odors (Litvinova & Vasilieva, 2004b). Consistent with this possibility is the fact that *Phodopus* species also engaging in "sand bathing" during which they roll their backs on loose substrate, which is believed to be a form of scent-marking behavior (Wynne-Edwards et al., 1992). Subject sex had a significant effect on side grooming, with males being more likely to side groom than females. This might suggest that males rely on side grooming more than females for olfactory communication.

The effect of stimulus type on leg grooming was also only marginally non-significant, though the probability of grooming in a trial tended to be highest in response to opposite-sex odor. It is unclear if leg grooming serves to spread any communicative odors originating in the leg region. Leg grooming was defined in the current study as the mouthing of any side of the hind leg, thus it is also unclear which neighboring odor sources it may spread. The inner leg may be close enough to mid-ventral gland or ano-genital odors, while the outer leg may be closer to odors from the sides and back. Leg grooming may also function in spreading odors from the mouth like saliva or sacculus secretion. No studies in *Phodopus* hamsters have looked to see if leg odors function in communication.

The mean probability of foot grooming tended to be lowest in response to unscented cotton and highest for opposite-sex odor, but stimulus type did not have a significant effect on the statistical model and pairwise comparisons also found no significant differences. Foot grooming in the current study was defined as mouthing the hindpaw. Foot odor from the hindpaw was not used by *Phodopus campbelli* males to distinguish between sexes (Lai et al., 1996). Nevertheless, the evidence thus far does not rule out the possibility that foot grooming plays a role in olfactory communication in *Phodopus*. Observations of *Phodopus campbelli* in the field have led to the proposal that the dense fur of the forepaws and hindpaws in *Phodopus* species functions to gather odors from multiple sources via grooming and then deposit those odors in trails as the animals traverse the landscape (Wynne-Edwards, 2003; Wynne-Edwards et al., 1992). Additional studies are needed to look for possible

plantar gland secretions and to determine if body odors collect on the feet.

Grooming of the ano-genital area and sebaceous mid-ventral gland were not able to be measured separately due to the small stature of dwarf hamsters and the viewing angle at which trials were video recorded. Therefore, ventral grooming was defined in the current study as the grooming of any part of the ventral surface. Ventral grooming was highest in response to opposite-sex odor. In addition to the mid-ventral gland and ano-genital area, the ventral surface of the body may also contain odors from urine and feces. Ventral grooming may function to spread out or otherwise enhance odors from one or more of these sources, and this enhancement may function to attract a mate. For example, the ano-genital odors of male prairie voles and meadow voles of both sexes were more strongly preferred by the opposite sex when originating from odor donors that had groomed at high rates verses low rates (Ferkin et al., 2001, 1996). Odors from the ventral surface that may be spread or otherwise enhanced by self-grooming have been shown to be used by *Phodopus* species to distinguish between sexes, a crucial component in finding a mate. *Phodopus sungorus* males housed in cages outdoors during the summer breeding season preferred to sniff the odor of urine and feces from females over males (Feoktistova & Meschersky, 1999). *Phodopus sungorus* males housed indoors under a long photoperiod simulating the breeding season made more ano-genital scent marks in response to female than male urine and soiled wood shavings, which likely contain odor from several ventral sources (Feoktistova, 1994). In *Phodopus campbelli* housed under a long photoperiod, females spent more time sniffing the odor of opposite-sex urine and mid-ventral gland

secretion, while males spent more time sniffing the odor of urine, mid-ventral gland secretion, and the ano-genital area of females in post-partum estrus over males (Lai et al., 1996).

It could not always be determined which body part was being groomed due to the position of the subject with respect to the perspective of the video camera, so the grooming of unknown body parts was pooled for analysis. Unknown grooming tended to be directed towards lower body parts because grooming the parts of the head was generally visible regardless of the subject's position and grooming lower body parts sometimes meant that the subject was hunched over with their backs to the camera. Regardless, the pattern of self-grooming unknown body parts is similar to the others, and it does not seem that, had these grooming behaviors have been able to be assigned to different body parts, the results and conclusions would be drastically different.

The possibility that self-grooming in response to conspecific odor was an artifact of the moderate amount of contact subjects had with the stimulus cotton when using it to construct a nest is ruled out by three pieces of evidence. First, the probability of using the stimulus cotton to construct a nest was higher for unscented cotton than either same-sex or opposite-sex cotton, a pattern that is opposite of what would be expected if subjects were self-grooming to clean themselves after having made contact with the stimulus. Second, rerunning the analysis of grooming any body part while including nest building in the model yielded similar results to excluding nest-building. Third, nest building was found to have a significant effect on self-grooming any body part, but self-grooming was higher in trials without nest building

than with nest building, again, the opposite of what would be expected if self-grooming was a response to nest building.

Scent-marking is a strong indication that an animal is intending to communicate. The current study was not specifically designed to measure scent-marking and so substrate projections, the preferred targets of marking (Reasner & Johnston, 1987), were not provided. However, the subjects made due with what they had and scent marked their home cage's gnawing block and the stimulus cotton itself. Also, only one camera recording with a top-down view was used which precluded the ability to distinguish between making with the ano-genital area and mid-ventral gland, which would have been easier to determine from a side view. The subject's wood particle litter also remained in the cage during testing, so urine and fecal marking was not recorded. Nevertheless, many instances of suspected scent-marking behaviors were observed and one-third of subjects scent marked. Since many of these instances were ambiguous, I took a conservative approach and analyzed the dichotomous occurrence of scent-marking in a trial rather than analyzing counts of scent marks. Consistent with the hypothesis that self-grooming functions in olfactory communication, the pattern of scent-marking paralleled the pattern of self-grooming. Scent-marking was not observed in any trials with unscented cotton stimulus, and the probability of scent-marking in a trial was significantly higher when the cotton stimulus was scented by opposite-sex odor than same-sex odor. Furthermore, analysis of grooming any body part showed that self-grooming was significantly higher in trials with scent-marking than without scent-marking. An alternative explanation for

this pattern is that the stimulus odors induced scent-marking while scent-marking induced self-grooming in order to clean the body surface of particles picked up through the act of dragging the body across a substrate. However, the effect of stimulus type on self-grooming any body part remained significant even when scent-marking was added to the statistical model. Similarly, ventral grooming may have been induced by scent-marking because the ventral surface is the specific part of the body dragged over substrate during scent-marking. Again, this explanation is unlikely as the pattern of self-grooming in response to stimulus type remained the same, and the amount of time spent ventral grooming in trials with scent-marking was not different from trials without scent-marking.

While the higher scent-marking response to opposite-sex odor suggests an attempt at attracting a mate, it was unclear as to whether scent marks were made with mid-ventral glands or the ano-genital area, and the function of these marks may differ according to type and sex of the marker. The exact communicative functions of ano-genital scent-marking and mid-ventral gland scent-marking in *Phodopus* remains somewhat unclear (Feoktistova, 1994; Heisler, 1984b; Reasner & Johnston, 1987; Wynne-Edwards, 2003; Wynne-Edwards & Lisk, 1987b, 1987a, 1988; Wynne-Edwards et al., 1992). Therefore, any interpretation of the function of the marks observed in the current study in attracting a mate is speculative.

Ano-genital marking seems more likely to function in sexual communication given that it directly involves the sexual organs like the vagina and possibly preputial glands. It has been shown in the laboratory that *Phodopus campbelli* males can

distinguish between female estrus state based on vaginal odor (Lai et al., 1996) and that *Phodopus campbelli* and *Phodopus sungorus* females change their rate of marking (ano-genital, mid-ventral, or urine) over their estrus cycle (Wynne-Edwards & Lisk, 1987b). In the wild, *Phodopus campbelli* females are believed to use vaginal marks to inform neighboring males of their estrus state (Wynne-Edwards, 2003). Perhaps the high number of scent marks observed in the current study by females in response to male odor is mostly due to vaginal marks, indicating sexual interest. *Phodopus campbelli* males preferred sniffing the mid-ventral gland odor of females in post-partum estrus to the mid-ventral gland odor of males (Lai et al., 1996), so it is also possible that females in the current study also scent marked with their mid-ventral gland to attract a mate. *Phodopus sungorus* males made more ano-genital marks in response to soiled wood shavings of females than other males (Feoktistova, 1994), so perhaps the scent-marking by males in the current study were ano-genital marks directed at females to attract a mate. Alternatively or additionally, males may have used mid-ventral gland scent marks to attract a mate. *Phodopus sungorus* males made more mid-ventral gland scent marks in response to soiled wood shavings from females than clean controls (Feoktistova, 1994). *Phodopus campbelli* females prefer the odor of mid-ventral gland secretion of males over females, and males prefer to scent mark with their mid-ventral gland in areas with female odor compared to clean areas (Lai et al., 1996; Reasner & Johnston, 1987).

The mid-ventral glands of *Phodopus* species are significantly larger in males, suggesting that they are used more by males than females for scent-marking (Heisler,

1984b; Reasner & Johnston, 1987). Observations of *Phodopus campbelli* in the field suggest that males use mid-ventral glands to mark territory boundaries with other males (Wynne-Edwards, 2003; Wynne-Edwards et al., 1992). Consistent with this, *Phodopus sungorus* males in the laboratory scent mark with their ventral gland much more in response to soiled wood shavings from males than from females or clean controls (Feoktistova, 1994). In the current study, a higher proportion of males may have scent marked in response to same-sex odor than females because those marks were mid-ventral gland marks meant to reaffirm territorial ownership of their home cage. Although, female *Phodopus sungorus* in the wild also scent mark along territorial boundaries with other females (Wynne-Edwards, 2003).

Overall, the pattern of self-grooming, investigation, and scent-marking behaviors in response to opposite-sex odor versus same-sex odor and unscented controls seemed to be stronger for female subjects than male subjects. This may be due to female subjects being more interested than male subjects in communicating with the opposite sex, as supported by the significantly higher investigation times observed for female subjects and a higher proportion of female subjects that scent marked in a trial. Interest by males in several different female odors can vary over the female's estrus cycle (Lai et al., 1996; Wynne-Edwards & Lisk, 1987b). In the current study, the estrus state of female odor donors was unknown, so male subjects may have experienced variation in the attractiveness of female odors they were exposed to (Ferkin, 2006), leading to a tendency for lower self-grooming rates than females in trials with opposite-sex odor. Even though stimulus cotton was left in an odor donor's

cage for several days, likely accumulating odors across a female's estrus cycle, I speculate that only the freshest odors conveying estrus state are likely to influence male behavior. Moreover, females may vary in their attraction to male odor depending on their estrus state and the estrus states of female subjects were also unknown. Self-grooming by females in response to opposite-sex odor may have been as high as it was due to a large number of female subjects being in estrus. Despite the pattern of a weaker self-grooming by males than females in response to opposite-sex odor, duration of self-grooming positively correlated with the duration of investigation in males, but not females, when analyzing sexes separately for the opposite-sex condition. Even though females on average were more interested in and self-groomed more in response to opposite-sex odor, this interest and self-grooming response did not correlate within trials.

The only other study that I am aware of to examine the self-grooming response of *Phodopus sungorus* to conspecific odors found more support against the hypothesis that self-grooming functions in olfactory communication than support for it. Feoktistova (1994) found that males self-groomed (over the ears and around the body, N.Y. Feoktistova, personal communication, 20 September 2014) more in an arena containing a small sample of mid-ventral gland secretion from same-sex odor donors than they did in a clean arena, and there was no difference in self-grooming between opposite-sex and same-sex conditions or opposite-sex and clean conditions. Furthermore, there were no differences in either of two types of self-grooming (rubbing face with forepaws, or over the ears and around the body, N.Y. Feoktistova,

personal communication, 20 September 2014) in response to urine, sacculus secretion, or soiled bedding of either same-sex, opposite-sex, or clean stimulus conditions (Feoktistova, 1994). These results weakly suggest that *Phodopus sungorus* males use one type of self-grooming to communicate with the same sex and do not use self-grooming to communicate with the opposite-sex. This finding is in direct opposition to the current study that found consistent evidence that males (and females) prefer to self-groom several body parts in response to opposite-sex odor over both same-sex odor and unscented controls. There are several notable differences in methodology between these studies that I am aware of and that may have contributed to the differences of findings, including that in the current study, 30 male subjects (and 30 females) were tested versus 10, males were sexually naïve, stimulus odors were presented as used cotton nest material and not soiled wood shavings, and subjects were tested in their home cage and not an open neutral arena. There are also several aspects of the methods used by Feoktistova (1994) that were not reported, so it would be difficult to try to explain the differences of our findings with direct comparisons. Nevertheless, I believe that the current study was a strong test of the hypothesis that self-grooming functions in olfactory communication for several reasons, including the use of detailed video review allowing for the measuring of grooming separate body parts, testing male and female subjects under each stimulus condition in a balanced random order, and using advanced statistics to account for repeated measures.

Self-grooming in response to conspecific odors has also been examined in the other *Phodopus species*. *Phodopus campbelli* males did not differ in the amount of

self-grooming (defined as facial, ventral, and flank grooming) in response to a clean arena or an arena recently temporarily occupied by a same-sex conspecific or an opposite-sex conspecific (Reasner & Johnston, 1987). *Phodopus roborovskii* males, also studied by Feoktistova (1994), self-groomed (rubbing the face with forepaws, N.Y. Feoktistova, personal communication, 20 September 2014) more in an arena containing a small sample of urine from an opposite-sex donor than a same-sex donor or in a clean arena. Furthermore, there were no differences in either of the two types of self-grooming she recorded (see above) in response to mid-ventral gland secretion or soiled wood shavings in either same-sex, opposite-sex or clean stimulus conditions (Feoktistova, 1994). Additional experimentation on *Phodopus* species is needed to determine if these differences in self-grooming behavior are genuine species differences or are due to methodological differences between studies.

In summary, the current study has found support for the hypothesis that self-grooming functions in olfactory communication in males and females of *Phodopus sungorus* and that this communicative function is likely to attract a mate. Communication has been defined in a variety of ways, but common elements that are relevant to the study of naturally selected animal behaviors include a sender of a signal and a receiver of the signal that alters its behavior in a way that provides fitness benefits to the sender. Additional studies are needed to examine how the receivers respond to the signals sent by self-grooming and how these responses benefit the groomers. Nevertheless, examining the differential production of this potential signal is an important first step and evidence is mounting that self-grooming functions in

olfactory communication.

This study adds to the growing number of studies in other species that have found support for a communicative function for self-grooming (for a recent review see Ferkin & Leonard, 2010). Given that self-grooming is such a widespread behavior among vertebrates, it is possible that self-grooming plays an important role in communication in many species. Additional carefully controlled studies are needed to examine this phenomenon on all levels of analysis: the mechanisms by which self-grooming enhances communication, the ontogeny of self-grooming, the functions of self-grooming, and the phylogenetic origins and relationships of the evolution of this behavior.

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CHAPTER 3

DOMINANCE-BASED ODOR DISCRIMINATION IN THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*), A EUSOCIAL MAMMAL

ABSTRACT

Naked mole-rats are atypical among mammals in their extreme form of eusocial cooperative breeding, and social dominance plays important roles in their reproductive division of labor, cooperation, mate choice, and access to food. Therefore, it is likely that natural selection has favored the evolution of mechanisms for individuals to discriminate between fellow colony members based on dominance. Since naked mole-rats are nearly blind, live underground, and are highly attuned to odor, it is also likely that the use of body odor is one such mechanism for discrimination based on dominance. This study tests the hypothesis that naked mole-rats can discriminate between the odors of fellow colony members based on the relative dominance rank of the odor donors. In a repeated measures design, subjects of three colonies of naked mole-rats were tested using a T-choice apparatus in which each stimulus arm contained the whole-body odor of one of two fellow colony members that differed in dominance rank and choice was measured by stimulus arm entry. Subjects were tested with several pairs of stimulus odor donors that varied in dominance status, sex, breeding status, and body weight, and subjects were analyzed separately according to sex, breeding status, absolute dominance rank, and relative dominance rank. The overall results were consistent: naked mole-rats can discriminate

between the odors of fellow colony members based on dominance, subordinate odors were never preferred, and dominant odors were mostly preferred. Additional questions generated by these results are discussed, including the identity of the odor sources that convey dominance, how these odors convey dominance, how the ability to discriminate between odors based on dominance helps to maximize fitness, and why the odors of dominant donors are preferred.

INTRODUCTION

Naked mole-rats (*Heterocephalus glaber*) are atypical among mammals in their extreme form of eusocial cooperative breeding (Jarvis, 1981), making them an excellent study system for investigating how individuals with the potential to reproduce inhibit their reproduction while living in cooperative groups that help a small minority of dominant reproductive conspecifics to rear young. A core principle of evolutionary theory is that individuals are selected to behave in ways that maximize their own reproductive fitness (Dawkins, 2006), so the existence of cooperatively breeding species in which individuals forgo their own reproductive efforts and instead help a conspecific to reproduce is a fascinating phenomenon that has intrigued evolutionary biologists since Charles Darwin (Darwin, 1909; Herbers, 2009). A great diversity of mammalian species are cooperative breeders (e.g., species of rodents, canids, herpestids, and primates) and they vary in the degree to which socially dominant individuals monopolize reproduction ("reproductive skew"; Bennett, 1994). The naked mole-rat is a eusocial, cooperatively breeding mammal (Jarvis, 1981) with

the most extreme level of reproductive skew (Lacey & Sherman, 1997). Naked mole-rats live in mixed-sex colonies of about 75 individuals on average with the largest group size recorded at 295 (Brett, 1991b), but there is a distinct reproductive division of labor with generally only a single dominant female (the "queen") and 1-3 dominant males producing offspring within the colony at any given time (Braude, 2000; Jarvis, 1981; Lacey & Sherman, 1991; Sherman, Jarvis, & Braude, 1992). Despite the fact that all adults have the potential to reproduce and that they have a weak inbreeding avoidance mechanism (Honeycutt, Nelson, Schlitter, & Sherman, 1991; H. K. Reeve, Westneat, Noon, Sherman, & Aquadro, 1990), subordinate non-breeders abstain from reproduction and less than 0.1% of non-breeders ever attain breeding status (Jarvis, O'Riain, Bennett, & Sherman, 1994). Several aspects of naked mole-rat biology contribute to the much higher lifetime reproductive success of a breeder than a non-breeder (Lacey & Sherman, 1997) including: lack of a non-breeding season (Brett, 1991b; Jarvis, 1991), large litter sizes (mea*N* = 12, maximum=28; Jarvis, 1991; Sherman, Braude, & Jarvis, 1999), and a relatively short inter-birth interval (range=72-84 d; Jarvis, 1991) considering their extreme longevity (maximum>32 y; Rodriguez et al., 2016) for a small rodent (mea*N* = 35 g).

Social dominance plays a central role in the reproductive division of labor and mate choice in naked mole-rats. Naked mole-rat colonies form linear dominance hierarchies with breeders being the most dominant individuals of their sex (Clarke & Faulkes, 1997, 1998; Schieffelin & Sherman, 1995). Given the great direct fitness benefits inherent with being a breeder, optimal skew theory predicts that individual

naked mole-rats will battle intensely over reproductive status (Keller & Reeve, 1994) and, indeed, fights to become a dominant breeder can be deadly (Clarke & Faulkes, 1997, 1998; Jarvis, 1991; Lacey & Sherman, 1991; Margulis, Saltzman, & Abbott, 1995). Fighting primarily takes place between females vying to become the queen, generally following the previous queen's absence (e.g., Clarke & Faulkes, 1997). Males generally do not fight with other males when a breeder male is removed (Clarke & Faulkes, 1998; Jarvis, 1991; Lacey & Sherman, 1991), possibly because females control mating by soliciting sex from males (Jarvis, 1991) and exercising mate choice (Ciszek, 2000; Clarke & Faulkes, 1998, 1999). However, females choose to mate with males that are among the most dominant, so male dominance status seems to indirectly influence breeding status (Clarke & Faulkes, 1998), and male-male fighting can also be high during new colony formation in the laboratory when dominance hierarchies are being established (Ciszek, 2000). Queens and females challenging to be queen may also attack males and even kill them, possibly in an attempt to suppress males that they are not interested in mating with but are showing physiological signs of being reproductive (Ciszek, 2000; Clarke & Faulkes, 1997; Jarvis, 1991; Lacey & Sherman, 1991) and/or because these males are supporting a rival female who is willing to mate with them (Ciszek, 2000; Lacey & Sherman, 1997).

Stable dominance hierarchies appear to be important for smooth transitions between dominant breeders and for maintaining cooperation in colonies of naked mole-rats. The extent to which a colony is socially unstable during a transition to a new queen varies. Rough transitions are marked by high levels of aggression, injuries,

and death; can involve fighting among every individual in the colony; and may lead to colony fission (Clarke & Faulkes, 1997; Jarvis, 1991; Lacey & Sherman, 1997; Margulis et al., 1995). More commonly observed are transitions in which only a small proportion of individuals, among the most dominant, are involved in aggression until dominance is established by a new queen (Clarke & Faulkes, 1997; Jarvis, 1991; Margulis et al., 1995). In smooth queen transitions, one of the most dominant females assumes breeding status and maintains dominance over all other would-be challengers without the escalation of aggression to the point of injury or death (Jarvis, 1991). When a breeder male becomes absent from the colony, generally there is no escalation of aggression (Clarke & Faulkes, 1998; Lacey & Sherman, 1991). Despite the potential for high levels of aggression to compete for breeding status, colonies can go through long periods of social stability with a dominant queen during which other females do not challenge the queen and injurious aggression is rare (Jarvis, 1991). During these stable times, the colony can spend more time on cooperative activities such as excavating new tunnels, maintaining old tunnels, retrieving food, and rearing young (Lacey, Alexander, Braude, Sherman, & Jarvis, 1991; Lacey & Sherman, 1991). For example, Jarvis (1991) reports maintaining in the laboratory two colonies that went for eight consecutive years without violent aggression, three that went for 12 years, and one that went for 13 years.

Dominance relationships also influence an individual's access to food. Despite the fact that naked mole-rats recruit fellow colony members to food sources (Judd & Sherman, 1996) and bring food back to the nest for sharing (Lacey & Sherman, 1991),

Schieffelin and Sherman (1995) found that individuals in hungry colonies would have tugging contests over pieces of food, revealing feeding hierarchies with dominant breeders at the top.

Since social dominance plays significant roles in several aspects of naked mole-rat inclusive fitness, natural selection has likely favored the evolution of mechanisms for individuals to discriminate between fellow colony members based on dominance. Naked mole-rats live in underground tunnel systems (Brett, 1991a) and are nearly blind (Hetling et al., 2005), so mechanisms for discriminating based on dominance are likely to be in the auditory or chemical (olfaction of volatiles or vomerolfaction of non-volatiles) modalities. They have several unique vocalizations (Judd & Sherman, 1996; Pepper, Braude, Lacey, & Sherman, 1991) and may be able to discriminate between individuals of different dominance rank based on "soft chirp" vocalizations (Yosida & Okanoya, 2009). Naked mole-rats are also highly attuned to social odors. For example, individuals discriminate between the odor of soiled bedding or used nesting material of their own colony versus another and will even attack a fellow colony member that had been scented with odors from a foreign colony (O'Riain & Jarvis, 1997). Additionally, queens discriminate between fellow colony members based on their level of relatedness, preferentially shoving those less related (Reeve & Sherman, 1991). Therefore, it is also likely that the use of body odor is one such mechanism for discrimination based on dominance.

There is evidence that other rodent species have the ability to discriminate between the odors of conspecifics based on dominance. Female bank voles

(*Cleithrionomys glareolus*) prefer the odor of urine from unfamiliar dominant males over the odor of urine from unfamiliar subordinate males (Hoffmeyer, 1982; Kruczek, 1997). Female brown lemmings (*Lemmus trimucronatus*; Huck, Banks, & Wang, 1981) and female golden hamsters (*Mesocricetus auratus*; White, Fischer, & Meunier, 1984) prefer the whole-body odor of unfamiliar dominant males over unfamiliar subordinate males. Male Norway rats prefer the whole-body odor of unfamiliar subordinate males to unfamiliar dominant males (Krames, Carr, & Bergman, 1969). Male guinea pigs (*Cavia porcellus*) react more aggressively to perineal gland odor of familiar dominant males than familiar subordinate males (Drickamer & Martan, 1992).

The primary goal of this study was to test the hypothesis that naked mole-rats can discriminate between the odors of fellow colony members based on the dominance status of the odor donors. In a repeated measures design, subjects of three colonies of naked mole-rats were tested using a T-choice apparatus in which each stimulus arm contained the whole-body odor of one of two fellow colony members that differed in dominance rank. To see if the characteristics of subjects influence dominance-based odor discrimination, subjects were analyzed separately according to sex, breeding status, absolute dominance rank, and relative dominance rank. To see if the characteristics of stimulus odor donors influence dominance-based odor discrimination, subjects were tested with several pairs of stimulus odor donors that varied in dominance status, sex, breeding status, and body weight. It was predicted that patterns of turning preference would emerge to indicate discrimination based on dominance, but the direction of preference (for dominant or subordinate) and the

specific patterns of preference based on subject and stimulus characteristics were not predicted in advance. The overall results were consistent: naked mole-rats can discriminate between the odors of fellow colony members based on dominance, subordinate odors were never preferred, and dominant odors were mostly preferred.

METHODS

Animal husbandry

Three colonies of naked mole-rats (*Heterocephalus glaber*) totaling 40 individuals were used in this study. Animals were housed in the laboratory of Dr. Paul Sherman (Mudd Hall, Department of Neurobiology and Behavior, Cornell University, Ithaca, NY). Each colony was housed in a separate tunnel system of clear polycarbonate tubes (5 cm diameter), acrylic boxes (20 x 20 x 12 cm, L x W x H), and polyvinyl chloride (PVC) plumbing joints. The tubes and boxes were transparent and the joints had clear plastic windows to allow for observation. Because naked mole-rats are a fossorial species, the colonies were housed without white light in two separate climate-controlled rooms (~28°C, ~40% RH). To reduce disturbances, tunnel systems rested upon foam-lined plywood suspended on inflated rubber inner tubes. Red desk lamps with incandescent bulbs were positioned a few inches above parts of the colony, including the nest chamber, to provide additional heat and to allow for observation. Naked mole-rats obtain water from their food. A mixture of fresh fruits and vegetables was provided fresh daily such that some food remained unfinished the following day. The most commonly provided food items were sweet potatoes, carrots, green beans,

lettuce, banana, pear, and apple. Occasional food items included jicama and grapes. Naked mole-rats usually used one chamber of their tunnel system as a latrine which was cleaned and furnished with aspen wood shavings daily. Some clean shavings were removed by the mole-rats to line their nest chamber.

The identity of individuals was tracked over the course of the study period by a combination of body weight, toe clippings, and distinguishing body characteristics such as tattoo scars, tail length, and overall body shape. To allow for easy observation, a black marker was used to write a letter or number on each individual's dorsal surface and was re-applied as needed, usually to all individuals of a colony on the same day.

Study animals

Colony "9300" (N = 15 total; 6 males; 9 females) was established in the laboratory of Dr. Rochelle Buffenstein (Department of Biology, The City College of New York, New York, NY) six years prior to the current study and transferred to Cornell University 114 days prior to the first behavioral trial of the current study. The queen of this colony was 11 years old and was the original founding queen. She was likely pregnant throughout most of the current study, although her pups did not survive for more than a few days. The rest of this colony is made up of three of her litters. When the colony arrived at Cornell University, the primary breeding male was the most dominant male of the colony, was seen to have sex with the queen on one occasion, and was the only male observed to engage in mutual ano-genital nuzzling with the queen, characteristic of a breeder (Ciszek, 2000; Faulkes, Abbott, Liddell,

George, & Jarvis, 1991; Jarvis, 1991; Lacey et al., 1991; Lacey & Sherman, 1991). This breeder male was the queen's 2-year-old son; naked mole-rats can show a high level of inbreeding (Reeve, Westneat, Noon, Sherman, & Aquadro, 1990). The previous mate to the queen (the breeder male's father) was not present in the colony. A second male, brother of the breeding male, was once seen attempting to copulate by unsuccessfully mounting the queen but died early in the study before its dominance rank could be determined. This male only contributed to a small portion of the conducted trials and is excluded from analyses requiring knowledge of his dominance status. The breeder male and his brother were born in the same litter as two other colony members (1 male; 1 female). Three months later, five other colony members were born (2 males; 3 females). Three months after that, the remaining five colony members were born (1 male; 4 females).

Colonies "1200B" and "1200D" were both formed by removing individuals from colony "1200" (not used in this study) and were founded at Cornell University. Due to a partial loss of breeding records, details such as breeding history, age, and relatedness are incomplete for these two colonies.

Colony "1200B" (N = 19 total; 9 males; 10 females) was established eight years prior to the current study. The best estimate from incomplete records is that the original breeding female of this colony died and that the last animals to be born and survive to adulthood in the colony were 6 years prior to the study. The female

identified as queen[1] of this colony was the only female to have a perforated vagina (Jarvis, 1991) and the only female to be observed engaging in mutual ano-genital nuzzling with the breeder male. Also, on three occasions during the study, the breeder male was observed to repeatedly attempt copulation with her exclusively but she was not observed to be receptive to his mounting. At the time of this study and for some years prior, this queen was the most dominant female in the colony despite being subordinate to four males, which is unusual for a queen. The breeder male of this colony was the founding breeder male, the most dominant individual in the colony, and was the only male ever seen attempting to copulate. Relatedness of individuals in this colony is unclear due to incomplete records, but it seems that all members were from at least five separate litters. Most members were born five to six years prior to the current study, and a few members, including the breeding male, were likely several years older. The lowest ranking individual was the youngest and had no surviving littermates during the current study.

Colony "1200D" (N = 6 total; 2 males; 4 females) was established four years prior to the current study. Relatedness and ages are unknown for this colony. There is no record of pregnancy or birth of pups for the female designated as Queen for this colony during the current study, but she was the most dominant individual, had a

[1] Although records are unclear, this female seems to have never become pregnant prior to the study. During the study, no female in the colony ever showed a weight change suggesting pregnancy. The lack of reproduction of this colony could be due in part to the laboratory going through a period of low breeding attempts and few successes across all colonies likely due to environmental conditions: lower than ideal temperatures for mating and lower than ideal humidity for successful rearing of pups, which was discovered and remedied after the study. Nevertheless, after the current study, this female apparently died before ever giving birth to a litter. Colonies in which a breeder female has maintained dominance but stopped reproducing has been reported before (Jarvis, 1991).

perforated vagina, and was observed to engage in mutual ano-genital nuzzling with only the most dominant male, who was consequently designated as the breeder male. It is unclear when this female first became queen, but she was not the queen two years prior to the current study. One non-breeder female was presumed to be particularly immature based on her small body size (~12 g) and relatively reduced mobility, so she was excluded from data collection.

Determining dominance hierarchies

Dominance hierarchies were determined separately for each colony by determining the dominance relationship between every two colony members. Overt aggression is uncommon except by breeders towards non-breeders in the form of shoving (Clarke & Faulkes, 2001; Jarvis, 1991; Lacey et al., 1991; Reeve & Sherman, 1991). When a subordinate is shoved by a dominant, the subordinate will often vocalize submission in the form of a "loud chirp" (*sensu* Pepper et al., 1991) which is easy for the human observer to detect as the caller's body will thrust forward with each call. On the basis of such casual observations alone, it was clear that the queens of colonies 9300 and 1200D and the breeder male of colony 1200B were the most dominant individuals in their colonies.

Most agonistic interactions, however, are subtle. When individuals approach each other in a tunnel and meet face-to-face, the two will briefly pause, quickly sniff, and the subordinate will often bow its head in submission to allow the dominant to pass over (Clarke & Faulkes, 1997, 1998, 2001). Passing behavior is not efficiently

observed when individuals are in their colony tunnel systems because it is very time-consuming to wait for every pair of animals to be walking toward each other with equal resolve and with no other colony members to get in the way. Therefore, passing behavior was elicited outside of the colony by removing two individuals and placing them into opposite ends of a Plexiglas tube (90 cm) and allowing them to walk toward each other and meet in the middle to pass over one another. Individuals were briefly held by the tail to elicit walking behavior and were released simultaneously. The tube was cut off-center into two pieces (60 cm, 30 cm) so that once the individuals passed each other and entered the other piece of the tube, the pieces could be repositioned end-to-end such that the individuals would be walking towards each other and once again meet in the middle to pass. This allowed for individuals to be systematically selected for repeated observations of passing behavior in a short period of time.

A successful passing observation was one in which an individual of a pair showed submission by actively lowering their head and bowing to the other as if cowering, not simply by being passed over by the other individual. This is because sometimes subordinates pass over dominants because they are eager to get away from them to avoid being shoved or because sometimes subordinates seem to have little patience for waiting for a slower-moving dominant to pass over them. In one session, a pair of individuals was made to pass each other several times before ten successful observations were made and it was clear which individual consistently bowed to the other or if individuals stopped reliably moving forward with equal resolve. Each pair of animals was tested in multiple sessions with at least a day separating sessions to

ensure that the dominance relationship was not being determined by one individual's lower resolve to move forward on a given day. When breeders were tested with this method, they often shoved subordinates who responded with submissive vocalizations thereby justifying an immediate end to the session.

Once every pair of individuals was tested in this way, a linear dominance hierarchy was revealed for each colony. Periodically throughout testing, the hierarchy was reconfirmed by testing every subsequent pair of individuals in a passing session. Naked mole-rat dominance hierarchies are very stable unless the colony is undergoing a period when multiple individuals are fighting to become breeders (personal observations), which did not occur during the current study. The linear dominance hierarchies at the end of the current study matched those first elucidated during the early part of the study.

Procedures

A T-choice apparatus was utilized to determine if naked mole-rats could discriminate between the odors of other members of their colony based on dominance and other demographic variables. Individuals entered the apparatus via a clear polycarbonate entry tube (25.4 cm) that led to a perpendicular PVC T-joint connecting to a pair of clear polycarbonate tubes (17.78 cm) that each had the odor of a different colony member. Choice arms terminated with PVC end caps. A choice was defined as an individual entering with all four paws and then taking one step forward into either the left or right arm of the apparatus. Each choice by an individual is referred to as a

"trial."

Beyond simply showing the ability to discriminate, it was the goal to see if a preference for certain odors existed in individuals that were behaving while in a state that approximated natural, undisturbed conditions. Therefore, subjects were not removed from the colony for testing, but instead given multiple opportunities to voluntarily enter the T-choice apparatus directly from their colony tunnel system. For each colony, the apparatus entry tube was attached to a heavily used thoroughfare of the colony's tunnel system to increase the chances that an individual would encounter the apparatus. Access to the entry tube was controlled with a series of removable shutters made up of layers of transparency paper (cellulose acetate) fixed together that could be slid into narrow slits in the tubes. The primary use of these shutters was to ensure that only one subject entered the apparatus at a time. The soft material of the shutter greatly reduced the chance that individuals were disturbed by the vibrations and sounds of putting one into position. If any individual showed signs of being disturbed by human action, they were prevented from entering the apparatus and free to return to the colony tunnel system until they calmed down and their behavior returned to normal. If a subject entered the apparatus but did not make a choice before exiting the apparatus, they were permitted to re-enter the apparatus repeatedly until making a decision after which they were prevented from entering for the same stimulus pair on the same day. Shutter slots were also sometimes positioned throughout the colony's tunnel system to block off remote areas and encourage targeted subjects to move in the direction of the apparatus entry point to increase the

efficiently of data collection. Voluntary choices made under these conditions should better reflect how naked mole-rats move around their tunnel systems in nature than would forced choices made under the condition of having been removed or forcibly isolated from their colony, a highly agitating condition in which the individual's main goal is to return to the colony.

Naked mole-rats are very sensitive to odor and can show hesitation to enter tubes that are clean and do not have the colony's odor. Pilot studies revealed that many subjects were more hesitant to enter the T-choice entry tube if it was unscented and those that did enter appeared to be uneasy and skittish. Additionally, if the choice arms of the apparatus were only scented by the odor donor, subjects appeared similarly uneasy and more hesitant to make a choice; some would even suddenly turn around and run out of the apparatus. Therefore, the apparatus entry tube and tubes of the choice arms were attached to the colony overnight (12-20 h) in order to acquire a base level of colony odor before being used in test trials. For odorization, the apparatus entry tube was connected in its testing position but, instead of terminating in the pair of choice arm tubes, terminated to the entry point of a network of tubes to be used as testing arms. Twenty stimulus tubes for the larger colonies (9300 and 1200B) and six for the smaller colony (1200D) were connected in a symmetrical configuration using PVC joints and end caps.

On a testing day, the network of colony-odorized tubes was detached from the colony tunnel system and dismantled into individual choice arm tubes that were shuffled in a pile on the counter. Two pairs of tubes were randomly chosen from the

pile to be used for each set of trials of a single stimulus pair on that day of testing. The two odor donors of a stimulus pair were individually isolated into separate stimulus tubes capped at both ends for five minutes so these tubes would acquire “whole-body” odor. After five minutes, the stimulus animals were transferred to the second pair of stimulus tubes and the first pair of tubes was positioned as the choice arms of the T-choice apparatus. During this second period of five minutes, the apparatus was open to a potential subject. Whether or not a subject entered the apparatus and made a choice, after the five minutes had elapsed, the first pair of stimulus arm tubes were detached from the apparatus and the stimulus animals were transferred from the second pair of stimulus tubes back to the first pair in their corresponding tubes to refresh their odor and to overlay odor that may have been left behind by the subject that briefly entered the tubes. This cycle repeated every five minutes until either every other colony member had entered the apparatus and made a choice, until 120 minutes had elapsed, or until individuals ceased entering the apparatus. When a subject made their choice and entered one of the stimulus arms, a shutter was put in place at the T-joint to briefly trap the subject in the end of the apparatus, which inevitably caused them to enter the non-chosen arm briefly before returning to the shutter. The shutter was then lifted to allow the subject to exit the apparatus and return to the colony. The subject was forced to briefly enter both arms of the apparatus so that any odor they may have contributed to the stimulus tubes simply by entering them would likely be balanced by entering both arms. Once a subject made a choice, the stimulus tubes were not used again until after their stimulus odor was refreshed by odor donors in the next 5-minute period.

Stimulus tubes were not reused for more than one stimulus pair on a given day of testing.

Stimulus tubes were odorized with "whole-body" odor by isolating a stimulus animal within. There are therefore several possible sources of odor that contributed to the odor of the stimulus tubes, including exudate from salivary glands, plantar glands, other skin glands, ano-genital glands, dry skin dander, urine, feces, and foreign particles stuck to the body surface (e.g., food or bedding material). After a stimulus animal was removed from a stimulus tube, any fecal pellets were also removed. Most fecal pellets were dry and easily shaken out of the tube. Moist fecal pellets were removed with a clean piece of paper towel, rubbing away fecal residue. If a fecal pellet was crushed by the stimulus animal's paws and spread throughout the stimulus tube or if the stimulus animal urinated in the tube, that tube was set aside for cleaning and not used for testing that day. The stimulus animal was then wiped with moist paper towel to prevent the spread of strong fecal or urine odor to another stimulus tube. Most colony members did not produce wet fecal pellets or urinate in stimulus tubes. Breeders, however, seemed to be particularly stressed by isolation and produced copious amounts of fecal pellets. Also, non-breeders hardly ever defecate or urinate when handled, yet breeders more readily do so. Therefore, when breeders were to be used as stimulus animals, they were first handled for several minutes until they ceased urinating and defecating after which they were placed into stimulus tubes.

After testing was complete for the day, all colony-odorized stimulus tubes, whether or not they were used for testing were washed with Sparkleen detergent

(Fisherbrand, ThermoFisher Scientific, Waltham, MA, U.S.A.) and hot water using a round scrub brush that fit inside the tubes, then rinsed with 50% ethanol followed by a rinse with deionized tap water. Tubes were dried by banging out most water and then drying with paper towel. The insides of tubes were not touched by human hands after cleaning and before testing. The apparatus entry tube and the PVC joints used to assemble the network of stimulus tubes for overnight colony odorization were also similarly cleaned daily before reassembly for another night of odorization.

Data for the entire study were collected over a 14-month period during which breeding statuses (breeder or non-breeder), dominance hierarchies (see above), and body weights (see below) remained consistent. This period of time is also a relatively small portion of a naked mole-rat's unusually-long (for a rodent) maximum lifespan during which they show little signs of aging (Buffenstein, 2005, 2008; Orr, Garbarino, Salinas, & Buffenstein, 2016; Sherman & Jarvis, 2002). For the first set of stimulus pairs tested it was the goal to test each subject in up to 16 trials per stimulus pair (colony 9300: ♂L–♂X, ♀9–♂I, ♀1–♀6; colony 1200B: ♂A–♂C, ♀7–♂O, ♀1–♀3, ♀6–♂J). In order to be able to test a wider variety of stimulus pairs in a shorter period of time, the goal number of trials was halved (8 trials) for the remaining stimulus pairs. Data for the maximum number of trials for each subject for each stimulus pair was not always collected because subject participation was voluntary, though all subjects had equal opportunities to participate. The number of subjects and total trials contributing to the dataset for each analysis are presented in the figures. An attempt was also made to balance which arm of the T-choice apparatus (left or right) contained

the odor of the dominant (versus the subordinate) odor donor. The left-right orientation of each stimulus pair was pre-determined for the day in random order across days. A subject was permitted to participate in one trial per stimulus pair per day and a colony was tested with up to five different stimulus pairs on the same day. Trails were conducted between 0900-2330 EST and before or after daily provisioning of food and latrine cleaning. Colony activity levels varied during testing but were not very predictable due to the fact that naked mole-rats do not show a colony-level circadian rhythm nor do individuals show circadian rhythms when housed with fellow colony members (Davis-Walton & Sherman, 1994).

For each colony, stimulus pairs were chosen to represent a diversity of donor odor combinations based on sex, weight, breeding status (breeder or non-breeder), and difference in dominance rank, while also minimizing reuse of particular individuals. When all else was equal, pairs were chosen randomly. Since some rank differences in a linear dominance hierarchy are few in number, some individuals ended up with greater representation than others as stimulus odor donors.

Weighing

Body weight was measured periodically throughout the study to see how it may influence olfactory discrimination and correlate with dominance rank. Healthy adult naked mole-rats in a colony comprised of individuals that are not challenging to become breeders show very consistent maintenance of body weight. This was true for all three colonies used in the current study. All members of a colony were weighed on

the same days.

Colony 9300 members were weighed five times spread over roughly an 11-month period that overlapped with testing of this colony. The mean weight of individuals of this colony during this time period, excluding the queen whose weight fluctuated with pregnancy, was 33.6 g (range: 23.3-43.8 g), while the mean weight change (increase or decrease) of these individuals was 3.0 g and the mean absolute weight change was 3.2 g. The queen's weight for this colony ranged from 49.2 (11 days after giving birth to a litter) to 63.2 (10 days prior to giving birth to a different litter).

Colony 1200B members were also weighed five times spread over an 11-month period that overlapped with testing of this colony. The mean weight of individuals of this colony during this time period was 44.6 g (range: 29.1-60.0 g), while the mean weight change (increase or decrease) during this period was 0 g with a mean absolute weight change of only 2.5 g.

Colony 1200D members were weighed twice over 2.5 months, first one day before testing began and then again four days after testing concluded. The mean weight of members of this colony during this time period was 35.5 g (range: 11.8-44.2 g). While the mean weight change (increase or decrease) during this period was 1.2 g and the mean absolute weight change was 1.3 g.

Statistical Analysis

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC,

U.S.A.). Statistical significance was set at $P < 0.05$ and marginally non-significant trends were noted when $0.05 \leq P \leq 0.10$.

The linear relationships between dominance rank and mean body weight for each colony were analyzed with two-tailed Spearman's rank-order correlations (PROC CORR, SAS) using the mean of each individual's body weight taken over the course of the study (see above).

Separate analyses of dominance-based odor discrimination were conducted for different subsets of subjects based on their characteristics (Table 3.1). Results were analyzed separately for each stimulus pair of each colony, pooled across stimulus pairs within a colony, or pooled across all stimulus pairs and colonies (Table 3.1). Some analyses of colony 1200D were excluded due to the very small number of subjects and low statistical power. Binary responses of turning left or right in the T-choice apparatus were analyzed using generalized estimating equations (GEE) logistic regression models with subject as a cluster variable and an exchangeable correlation structure within subjects to control for repeated measures (PROC GENMOD, SAS). A nested correlation structure of individual within stimulus pair was not necessary (or possible) in analyses of pooled data from multiple stimulus pairs, so only subject was specified in the repeated statement option. This is sufficient because the statement only needs to distinguish correlated observations (those from the same subject) from uncorrelated ones because the GEE method is robust ("SAS Usage Note 24200," 2004). Unless otherwise stated, the probability of turning right was modeled as the response to whether the right arm of the T-choice apparatus had the odor of the

Table 3.1. Categories of subject characteristics, subjects analyzed, colonies analyzed, whether or not stimulus pairs were analyzed separately or pooled together, and figures were results are presented for each analysis of dominance-based odor discrimination in three colonies of naked mole-rats (*Heterocephalus glaber*).

Subject characteristics	Subjects analyzed	Colonies analyzed	Stimulus pairs analyzed	Figure
sex	all subjects	pooled: 9300, 1200B, 1200D	pooled	3.3a
		9300	separately	3.4a
		1200B	separately	3.5a
		1200D	separately	3.6
	all females	separately: 9300, 1200B, 1200D	pooled	3.3b
		9300	separately	3.4b
		1200B	separately	3.5b
	all males	separately: 9300, 1200B, 1200D	pooled	3.3c
		9300	separately	3.4c
		1200B	separately	3.5c
breeding status	all non-breeders	pooled: 9300, 1200B, 1200D	pooled	3.7a
		9300	separately	3.8a
		1200B	separately	3.9a
	female non-breeders	separately: 9300, 1200B	pooled	3.7b
		9300	separately	3.8b
		1200B	separately	3.9b
	male non-breeders	separately: 9300, 1200B	pooled	3.7c
		9300	separately	3.8c
		1200B	separately	3.9c
	all breeders	pooled: 9300, 1200B, 1200D	pooled	3.10
	female breeders	pooled: 9300, 1200B, 1200D	pooled	3.10
	male breeders	pooled: 9300, 1200B, 1200D	pooled	3.10
absolute dominance rank	most dominant	separately: 9300, 1200B	pooled	3.11a
		9300	separately	3.12a
		1200B	separately	3.13a
	most subordinate	separately: 9300, 1200B	pooled	3.11b
		9300	separately	3.12b
		1200B	separately	3.13b
relative dominance rank	ranked between odor donors	separately: 9300, 1200B, 1200D	pooled	3.14a
		9300	separately	3.15a
		1200B	separately	3.16a
	ranked above or below odor donors	separately: 9300, 1200B	pooled	3.14b
		9300	separately	3.15b
		1200B	separately	3.16b

dominant or subordinate odor donor of the stimulus pair. An effort was made for each stimulus pair to balance the number of an individual's trials in which the odor of the dominant was on the left or right. To illustrate that the choice of modeling the probability of turning right or the probability of turning left was arbitrary and produced nearly identical results for the effect of stimulus dominance of the tube chosen, both analyses were conducted for comparison with trials pooled across stimulus pairs and colonies. Type III tests for significance of fixed effects were calculated using the default score statistics for GEE. Estimate LS-means are reported on the inverse linked scale as probabilities with 95% confidence intervals.

To see if the strength of the preference for the dominant odor of the stimulus pair was influenced by the dominance rank difference between the two odor donors, a GEE logistic regression model was used to model the effect of the difference in dominance rank of the odor donors on the probability of turning into the tube with the odor of the dominant donor. The finer details of the GEE model were otherwise the same as described above.

To control for multiple hypothesis testing of multiple stimulus pairs of a specific subject demographic within a colony, adjusted *P* values were calculated using the False Discovery Rate (FDR) option of PROC MULTTEST (SAS) following Benjamini and Hochberg (1995). Controlling for the FDR is a more balanced approach at reducing Type I and Type II statistical errors and is a preferred alternative to methods such as the Bonferroni adjustment that very conservatively controls for Type

I error at the expense of increasing Type II error and causes loss of power with small sample sizes (Nakagawa, 2004). For the current study, a false discovery rate of 0.05 was chosen, indicating that up to 5% of tests in which the null hypothesis was rejected, were done so in error, i.e., they were "false positives."

Ethical Note

The methods used were approved by Cornell University's Institutional Animal Care and Use Committee. At the end of the study, animals remained in the colony for use in other studies.

RESULTS

Does dominance rank correlate with body weight?

Yes. There was a significant negative correlation with dominance rank and mean body weight for all three colonies such that the more dominant a subject was the heavier they tended to be (Figure 3.1). The negative correlation remained significant for colony 1200D even if the smallest individual was excluded from analysis (Spearman's rank correlation: $r_s = -0.900$, $N = 5$, $P = 0.037$).

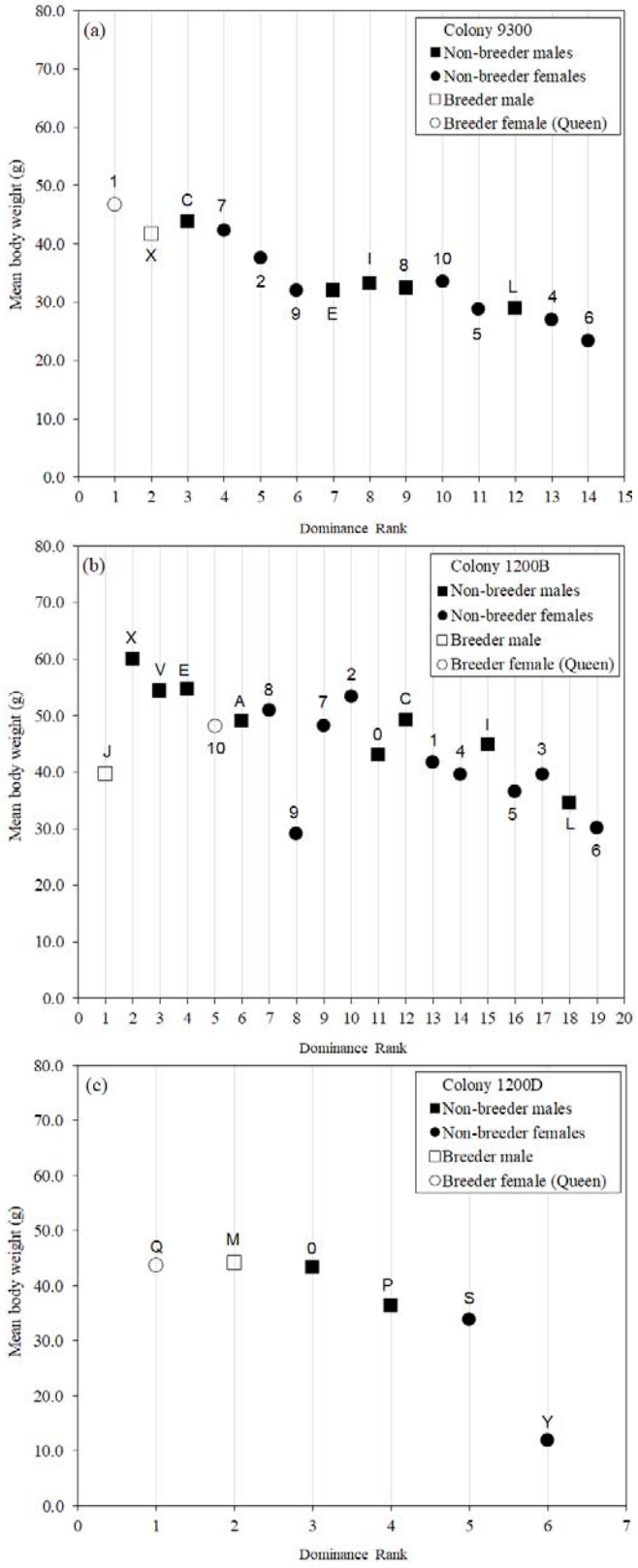

(a)
Colony 9300
Non-breeder males
Non-breeder females
Breeder male
Breeder female (Queen)
Mean body weight (g)
Dominance Rank
1
X
C
7
2
9
E
I
8
10
5
L
4
6
(b)
Colony 1200B
Non-breeder males
Non-breeder females
Breeder male
Breeder female (Queen)
Mean body weight (g)
Dominance Rank
J
X
V
E
10
A
8
9
7
2
0
C
1
4
I
5
3
L
6
(c)
Colony 1200D
Non-breeder males
Non-breeder females
Breeder male
Breeder female (Queen)
Mean body weight (g)
Dominance Rank
Q
M
0
P
S
Y

Figure 3.1. Mean body weight as a function of dominance rank for three colonies of naked mole-rats (*Heterocephalus glaber*) as analyzed with Spearman's rank correlation: (a) colony 9300 (r_s = -0.899, N = 14, P < 0.0001*); (b) colony 1200B (r_s = -0.660, N = 19, P = 0.002*); (c) colony 1200D (r_s = -0.943, N = 6, P = 0.005*). Dominance decreases as rank number increases such that the most dominant individual has a rank of 1. To provide a conservative analysis, the lowest body weight was used for the breeding female of colony 9300, taken 11 days after giving birth, instead of the mean because this female was usually pregnant when the colony was weighed. Filled squares indicate non-breeder males, open squares indicate breeder males, filled circles indicate non-breeder females, open circles indicate breeder females ("queens"). Letters and numbers labeling each data point indicate individual identifiers.

Do naked mole-rats show dominance-based discrimination?

Yes. Overall, subjects preferred to enter the stimulus arm that had the odor of the more dominant of two odor donors from the same colony.

When all of the T-choice data for the entire study were pooled across all of the three colonies and 35 stimulus pairs tested, the probability of turning into the right (versus left) stimulus arm was significantly higher if the right arm contained the odor of the more dominant of two odor donors from the same colony (logistic regression: $\chi^2_1 = 27.84$, $P < 0.000001$; Figure 3.2). Since the direction of the arm that contained the odor from the more dominant individual was balanced across trials, the choice to model the probability of turning right in subsequent analyses was arbitrary. To illustrate this point, the data were reanalyzed modeling the probability of turning into the left stimulus arm and nearly identical highly significant results were obtained (logistic regression: $\chi^2_1 = 27.84$, $P < 0.000001$; Figure 3.2).

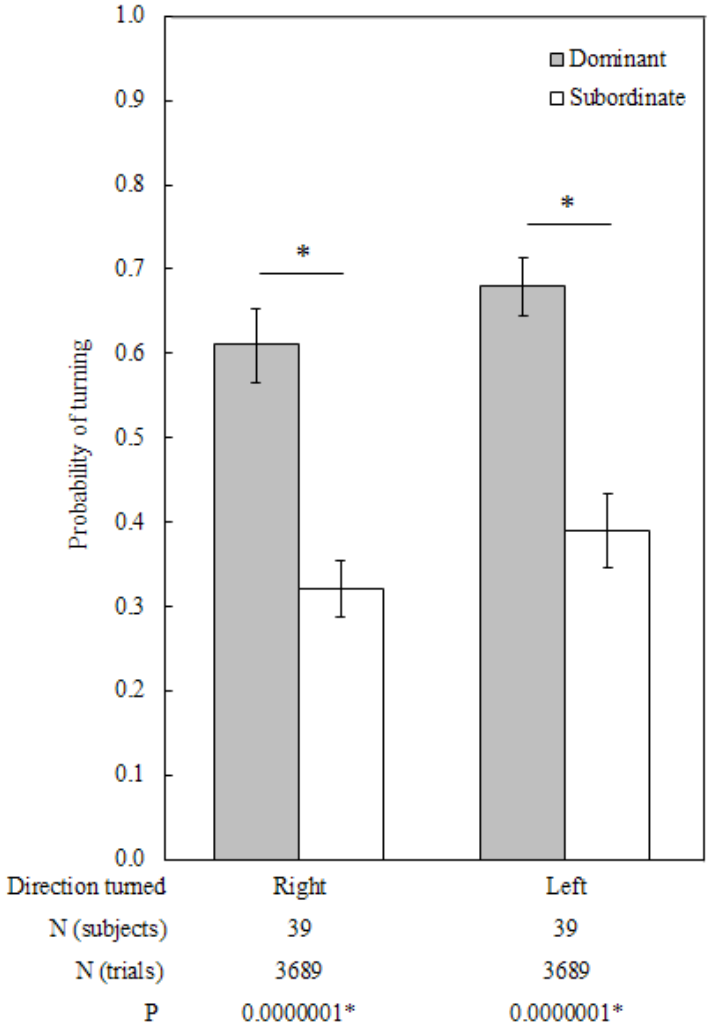
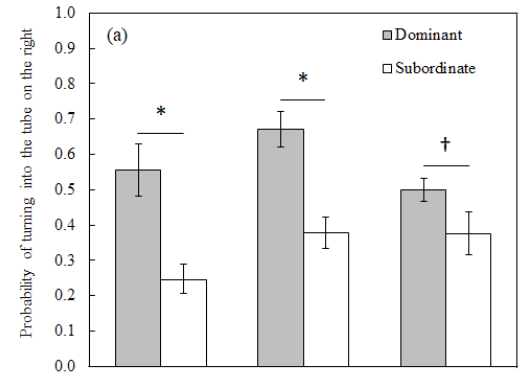
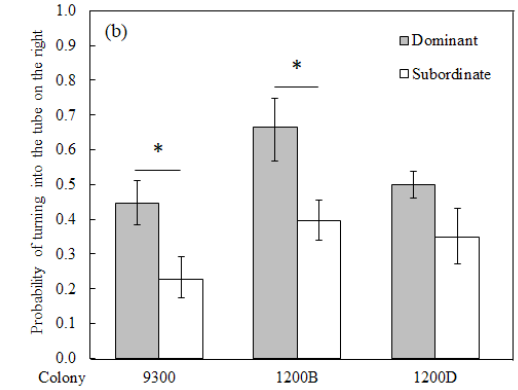


Figure 3.2. Probability of turning into the right or left arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members. These data are pooled across the entire study of three colonies (colony 9300: $N = 15$ subjects, colony 1200B: $N = 19$ subjects, and colony 1200D: $N = 5$ subjects) and across multiple stimulus pairs of odor donors (see text). The side that each odor type was on was balanced across trials. Separate GEE logistic regression models were used to model the probability of turning in each direction to illustrate that choosing which direction to model was arbitrary and yielded very similar results for examining the effect of the odor type on direction turned. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. "P" indicates the level of statistical significance. Statistically significant P values are denoted with an asterisk (*).

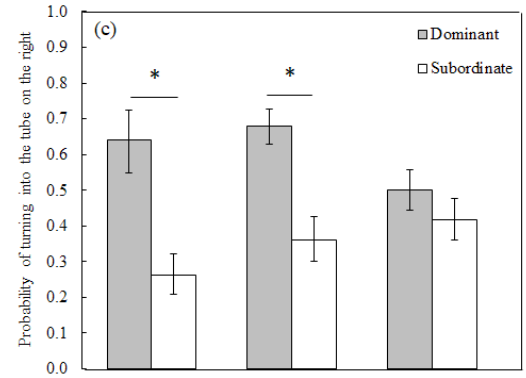
When the T-choice data were analyzed separately for each colony while pooling across all stimulus pairs tested, the probability of turning into the right stimulus arm was significantly higher if the right arm contained the odor of the dominant stimulus for both colony 9300 (logistic regression: $\chi^2_1 = 11.5$, $P_{adj} = 0.001$; Figure 3.3a) and colony 1200B (logistic regression: $\chi^2_1 = 15.41$, $P_{adj} = 0.0003$; Figure 3.3a). For colony 1200D, the probability of turning into the arm with the odor of the dominant stimulus was higher than for the subordinate stimulus, but this trend was marginally non-significant when all five subjects were analyzed together (logistic regression: $\chi^2_1 = 3.69$, $P_{adj} = 0.055$; Figure 3.3a). Colony 1200D was the smallest colony tested, i.e., it had the fewest number of subjects ($N = 5$), and thus suffered from low statistical power.



Colony	9300	1200B	1200D
N (subjects)	15	19	5
N (trials)	1658	1791	240
P	0.0007*	<0.0001*	0.0548†
P-adjusted	0.0011*	0.0003*	0.0548†



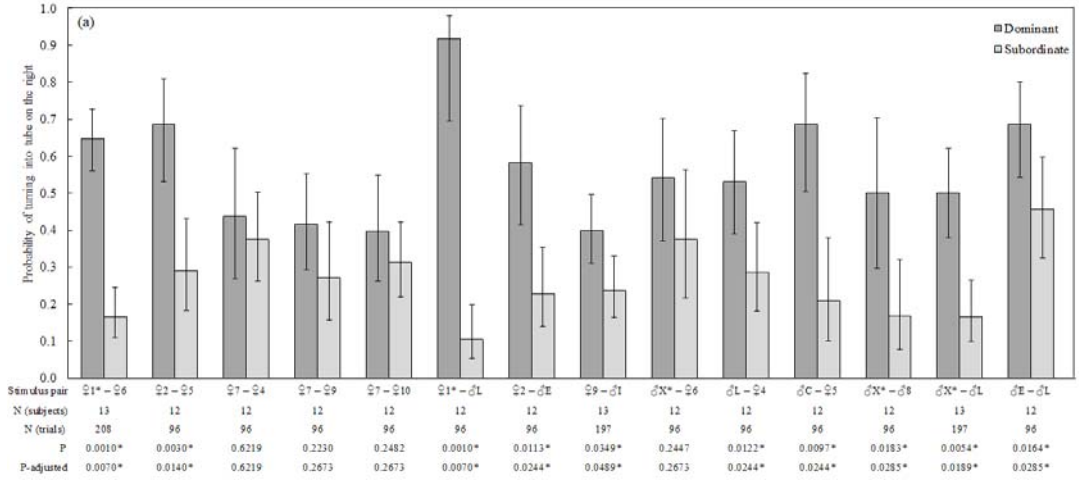
Colony	9300	1200B	1200D
N (subjects)	7	9	3
N (trials)	716	845	144
P	0.0324*	0.0107*	0.1011
P-adjusted	0.0486*	0.0321*	0.1011



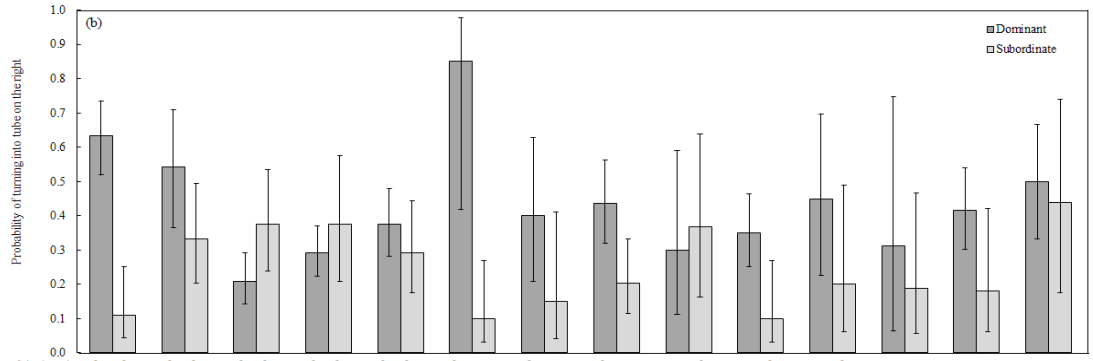
Colony	9300	1200B	1200D
N (subjects)	8	10	2
N (trials)	942	946	96
P	0.0069*	0.0028*	0.3173
P-adjusted	0.0104*	0.0084*	0.3137

Figure 3.3. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **three colonies** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using (a) **all subjects**, (b) male subjects only, or (c) female subjects only. Separate GEE logistic regression models were used for each colony while pooling across multiple stimulus pairs of odor donors (colony 9300: 14 stimulus pairs; colony 1200B: 11 stimulus pairs; and colony 1200D: 10 stimulus pairs). Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

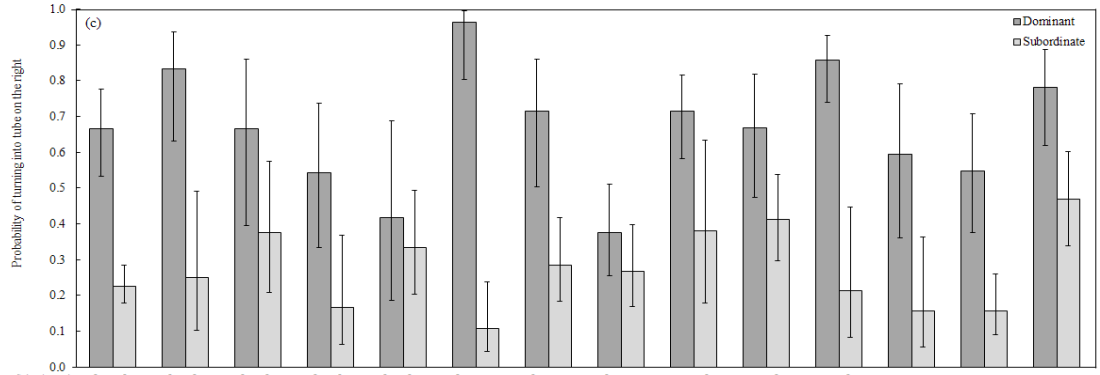
Separate analyses of each stimulus pair tested within each colony paint a similar picture. For colony 9300, the probability that all subjects turned into the stimulus arm that contained the odor of the dominant stimulus was higher for all 14 stimulus pairs tested with this difference achieving statistical significance in 10/14 (71%) stimulus pairs (Figure 3.4a). Similarly, for colony 1200B, the probability that all subjects turned into the stimulus arm that contained the odor of the dominant stimulus was higher for 10/11 (91%) stimulus pairs tested with this difference achieving statistical significance in 8/11 (73%) stimulus pairs (Figure 3.5a). For colony 1200D, the probability that all subjects turned into the arm that contained the odor of the dominant stimulus was higher for 7/10 (70%) stimulus pairs tested, although the difference in probabilities did not reach statistical significance for any of the 10 stimulus pairs tested (Figure 3.6). In none of the stimulus pairs tested across all three colonies was there a significant preference for the odor of the subordinate stimulus (Figures 3.4a, 3.5a, & 3.6).



Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	13	12	12	12	12	12	12	13	12	12	12	12	13	12
N (trials)	208	96	96	96	96	96	96	197	96	96	96	96	197	96
P	0.0010*	0.0030*	0.6219	0.2230	0.2482	0.0010*	0.0113*	0.0349*	0.2447	0.0122*	0.0097*	0.0183*	0.0054*	0.0164*
P-adjusted	0.0070*	0.0140*	0.6219	0.2673	0.2673	0.0070*	0.0244*	0.0489*	0.2673	0.0244*	0.0244*	0.0285*	0.0189*	0.0285*

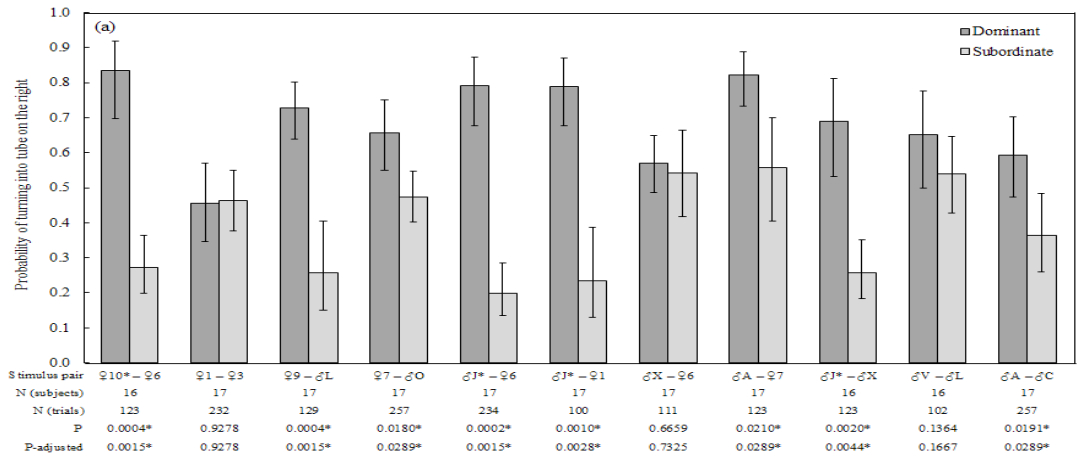


Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	7	6	6	6	6	5	5	6	5	5	5	4	5	4
N (trials)	107	48	48	48	48	40	40	85	39	40	40	32	69	32
P	0.0207*	0.0253*	0.1025	0.4142	0.4142	0.0469*	0.1967	0.1158	0.7451	0.0588†	0.2971	0.5271	0.1887	0.6547
P-adjusted	0.1771	0.1771	0.2702	0.5272	0.5272	0.2058	0.3442	0.2702	0.7451	0.2058	0.4622	0.6150	0.3442	0.7051

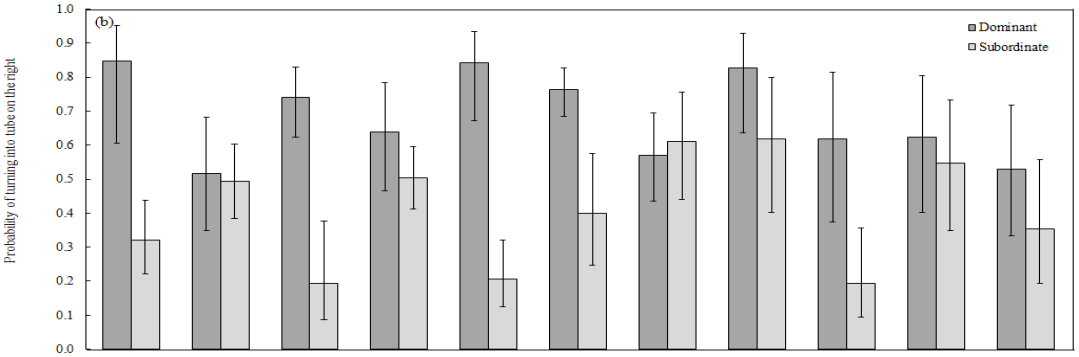


Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	6	6	6	6	6	7	7	7	7	7	7	8	8	8
N (trials)	101	48	48	48	48	65	56	112	57	57	57	64	128	64
P	0.0181*	0.0196*	0.2087	0.0833†	0.4142	0.0088*	0.0285*	0.1573	0.0879†	0.0675†	0.0162*	0.0196*	0.0138*	0.0124*
P-adjusted	0.0392*	0.0392*	0.2248	0.1119	0.4142	0.0392*	0.0499*	0.1835	0.1119	0.1050	0.0392*	0.0392*	0.0392*	0.0392*

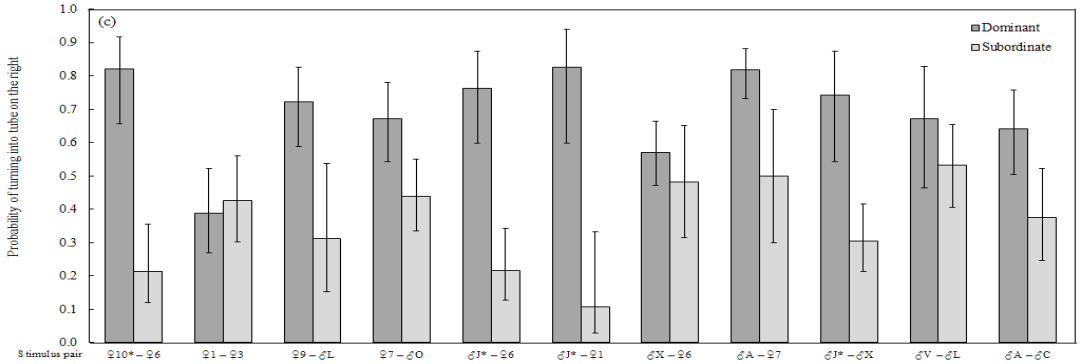
Figure 3.4. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 9300** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using (a) **all subjects**, (b) male subjects only, or (c) female subjects only. Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).



Stimulus pair	♀10* – ♀6	♀1 – ♀3	♀9 – ♂L	♀7 – ♂O	♂J* – ♀6	♂J* – ♀1	♂X – ♀6	♂A – ♀7	♂J* – ♂X	♂V – ♂L	♂A – ♂C
N (subjects)	16	17	17	17	17	17	17	17	16	16	17
N (trials)	123	232	129	257	234	100	111	123	123	102	257
P	0.0004*	0.9278	0.0004*	0.0180*	0.0002*	0.0010*	0.6659	0.0210*	0.0020*	0.1364	0.0191*
P-adjusted	0.0015*	0.9278	0.0015*	0.0289*	0.0015*	0.0028*	0.7325	0.0289*	0.0044*	0.1667	0.0289*



Stimulus pair	♀10* – ♀6	♀1 – ♀3	♀9 – ♂L	♀7 – ♂O	♂J* – ♀6	♂J* – ♀1	♂X – ♀6	♂A – ♀7	♂J* – ♂X	♂V – ♂L	♂A – ♂C
N (subjects)	9	9	8	8	8	8	8	8	7	7	7
N (trials)	67	125	58	129	107	48	54	58	52	43	104
P	0.0132*	0.8073	0.0080*	0.1034	0.0152*	0.0250*	0.6733	0.1967	0.0630†	0.5152	0.2316
P-adjusted	0.0557†	0.8073	0.0557†	0.1896	0.0557†	0.0688†	0.7406	0.3091	0.1386	0.6297	0.3185



Stimulus pair	♀10* – ♀6	♀1 – ♀3	♀9 – ♂L	♀7 – ♂O	♂J* – ♀6	♂J* – ♀1	♂X – ♀6	♂A – ♀7	♂J* – ♂X	♂V – ♂L	♂A – ♂C
N (subjects)	7	8	9	9	9	9	9	9	9	9	10
N (trials)	56	107	71	128	127	52	57	65	71	59	153
P	0.0113*	0.5594	0.0194*	0.0749†	0.0059*	0.0151*	0.2666	0.0524†	0.0120*	0.1648	0.0404*
P-adjusted	0.0415*	0.5594	0.0427*	0.1030	0.0415*	0.0415*	0.2933	0.0823†	0.0415*	0.2014	0.0741†

Figure 3.5. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 1200B** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using (a**) all subjects**, (b) male subjects only, or (c) female subjects only. Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

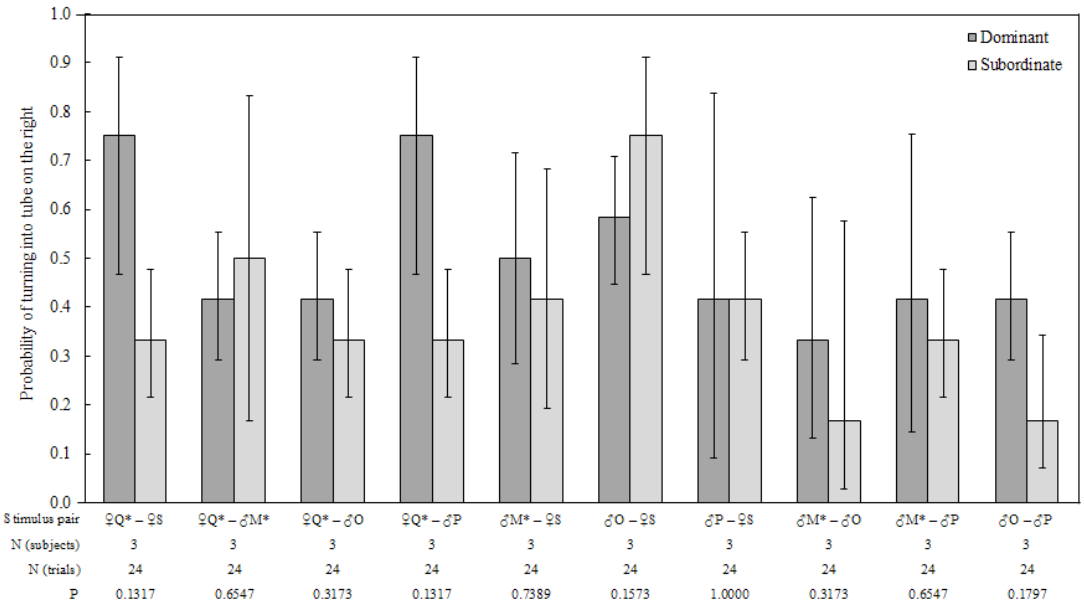


Figure 3.6. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 1200D** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using all subjects. Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. No results were statistically significant so it was not necessary to control for multiple hypothesis testing.

The results of the dominance-based discrimination tests were reanalyzed according to different subject characteristics (summarized in Table 3.1) to answer the questions below.

Does dominance-based discrimination depend on subject's sex?

No. Turning preferences were reanalyzed separately for male and female subjects and the overall pattern of results for each sex was consistent with the analyses of both sexes combined, i.e., both males and females showed a preference for the odor of the more dominant stimulus.

For male subjects, when the T-choice data were analyzed separately for each colony while pooling across all stimulus pairs tested, the probability of turning into dominant stimulus arm was significantly greater for both colony 9300 (logistic regression: $\chi^2_1 = 4.58$, $P_{adj} = 0.049$; Figure 3.3b) and colony 1200B (logistic regression: $\chi^2_1 = 6.51$, $P_{adj} = 0.032$; Figure 3.3b). Similarly, for females subjects, the dominant stimulus odor was preferred for both colony 9300 (logistic regression: $\chi^2_1 = 7.31$, $P_{adj} = 0.010$; Figure 3.3c) and colony 1200B (logistic regression: $\chi^2_1 = 8.91$, $P_{adj} = 0.008$; Figure 3.3c). For both sexes of colony 1200D, the probability of choosing the dominant stimulus was greater than the probability of choosing the subordinate stimulus, but these differences were not significant for either sex when analyzed separately, due to low statistical power (Figures 3.3b & 3.3c).

Analyses of each stimulus pair tested within each of the two larger colonies were conducted separately for each sex, but doing so reduced statistical power.

Nevertheless, as is the case for all subsequent separate analyses of subjects by sex, the results are consistent overall. For no analysis was there a statistically significant preference for the subordinate stimulus. For male subjects of colony 9300, the dominant stimulus was preferred in 11/14 (79%) stimulus pairs, although no difference was significant after controlling for false discovery (Figure 3.4b). For female subjects of colony 9300, the dominant stimulus was preferred in all 14 stimulus pairs, and for 8/14 (57%) stimulus pairs that difference remained statistically significant after controlling for false discovery (Figure 3.4c). For male subjects of colony 1200B, the dominant stimulus was preferred in 10/11 (91%) stimulus pairs, but for only four stimulus pairs was this difference marginally non-significant (Figure 3.5b). For female subjects of colony 1200B, the dominant stimulus was preferred in 10/11 (91%) stimulus pairs, and that difference remained statistically significant for 5/11 (46%) stimulus pairs and marginally non-significant for 2/11(18%) stimulus pairs after controlling for false discovery (Figure 3.5c).

See below for analyses comparing breeder and non-breeder subjects of each sex and a summary of how subjects of each sex responded to different stimulus pair sex combinations.

Does dominance-based discrimination depend on subject's breeding status?

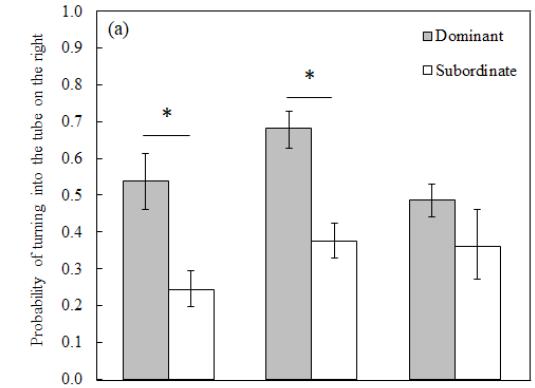
It seems no, but analysis of a higher sample of breeders is warranted. Turning preferences were reanalyzed using non-breeder subjects of both sexes and separately for each sex, and the overall pattern of preference for the dominant stimulus odor was

consistent across analyses. Breeder males and queens also followed this pattern, but differences were not significant, likely due to low statistical power of small sample sizes.

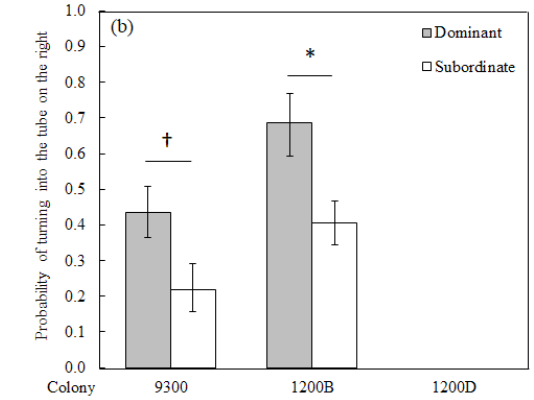
When the T-choice data were analyzed separately for each colony while pooling across all stimulus pairs tested, there was a significant preference for the dominant stimulus odor by non-breeder subjects for both colony 9300 (logistic regression: $\chi^2_1 = 9.96$, $P_{adj} = 0.002$; Figure 3.7a) and colony 1200B (logistic regression: $\chi^2_1 = 13.88$, $P_{adj} = 0.0006$; Figure 3.7a). For colony 1200D, the probability of the non-breeder subjects ($N = 3$) turning into the arm with the dominant stimulus odor was higher than for the arm with the subordinate odor, but this difference was not significant (Figure 3.7a). When analyzing male non-breeders, colony 9300 showed a marginally non-significant preference (logistic regression: $\chi^2_1 = 3.64$, $P_{adj} = 0.056$; Figure 3.7b) and colony 1200B showed a statistically significant preference for the dominant stimulus odor (logistic regression: $\chi^2_1 = 5.92$, $P_{adj} = 0.030$; Figure 3.7b). For female non-breeders, colony 9300 (logistic regression: $\chi^2_1 = 6.45$, $P_{adj} = 0.011$; Figure 3.7c) and colony 1200B (logistic regression: $\chi^2_1 = 7.97$, $P_{adj} = 0.009$; Figure 3.7c) showed a significant preference for the dominant stimulus odor.

For colony 9300, non-breeder subjects preferred the dominant stimulus odor in all 14 stimulus pairs and for 5/14 (36%) stimulus pairs this preference was statistically significant, while for 4/14 (29%) stimulus pairs this preference was marginally non-significant after controlling for false discovery (Figure 3.8a). For male non-breeders of colony 9300, in 11/14 (79%) stimulus pairs, the dominant stimulus odor was

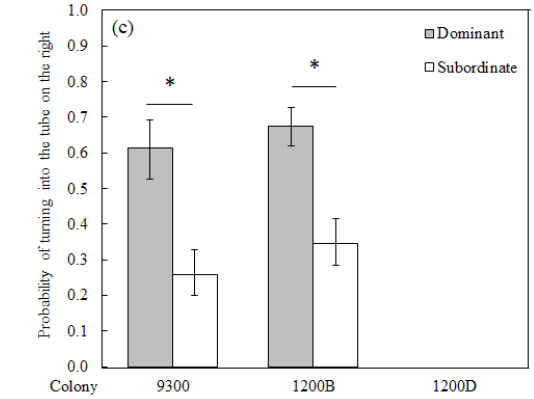
preferred, although no stimulus pair showed a significant preference after controlling for false discovery rate (Figure 3.8b). For female non-breeders of colony 9300, in 13/14 (93%) stimulus pairs the dominant stimulus odor was preferred and 8/14 (57%) this preference was marginally statistically significant after controlling for false discovery (Figure 3.8c).



Colony	9300	1200B	1200D
N (subjects)	12	17	3
N (trials)	426	1604	144
P	0.0016*	0.0002*	0.1599
P-adjusted	0.0024*	0.0006*	0.1599

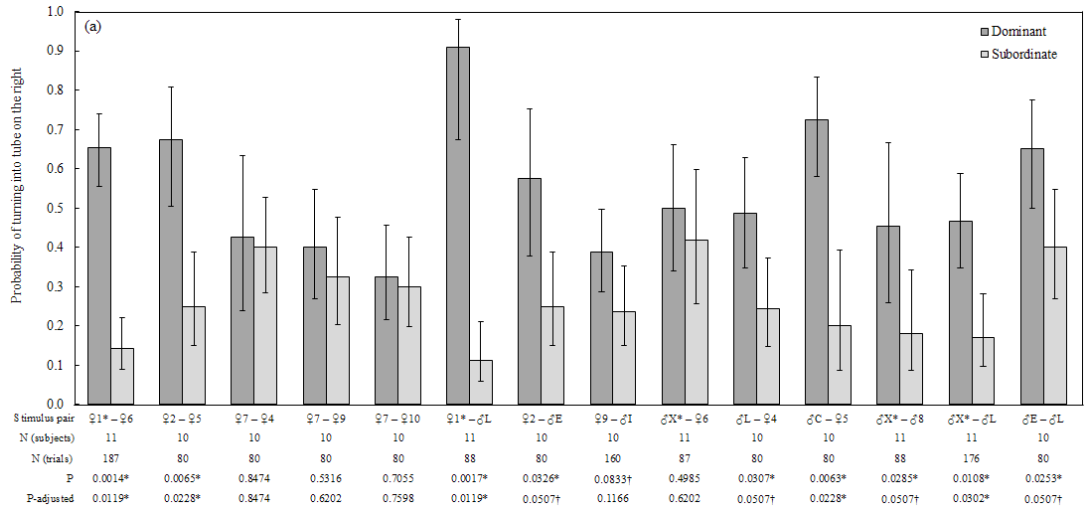


Colony	9300	1200B	1200D
N (subjects)	5	8	-
N (trials)	597	760	-
P	0.0564†	0.0150*	-
P-adjusted	0.0564†	0.0300*	-

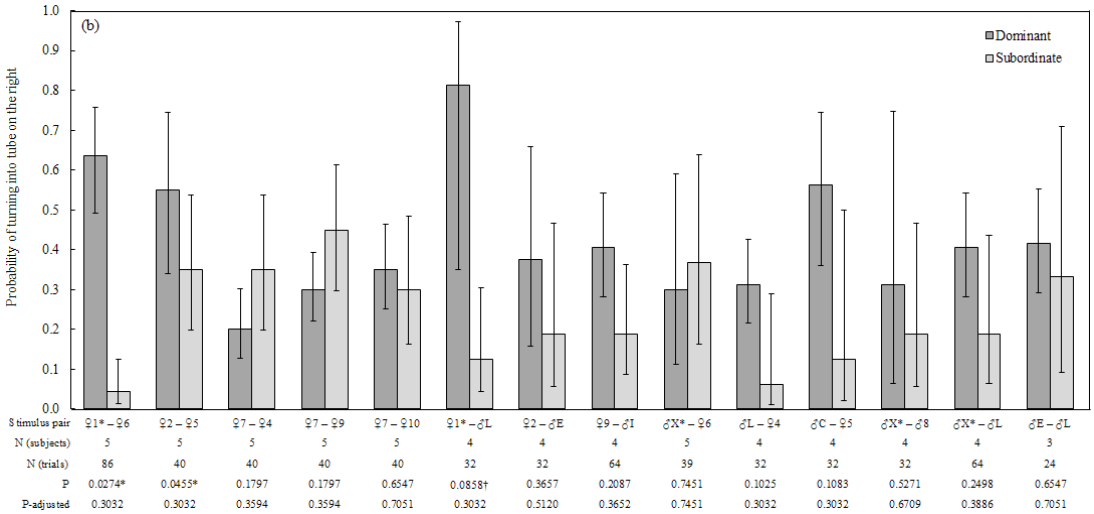


Colony	9300	1200B	1200D
N (subjects)	7	9	-
N (trials)	829	844	-
P	0.0111*	0.0047*	-
P-adjusted	0.0111*	0.0094*	-

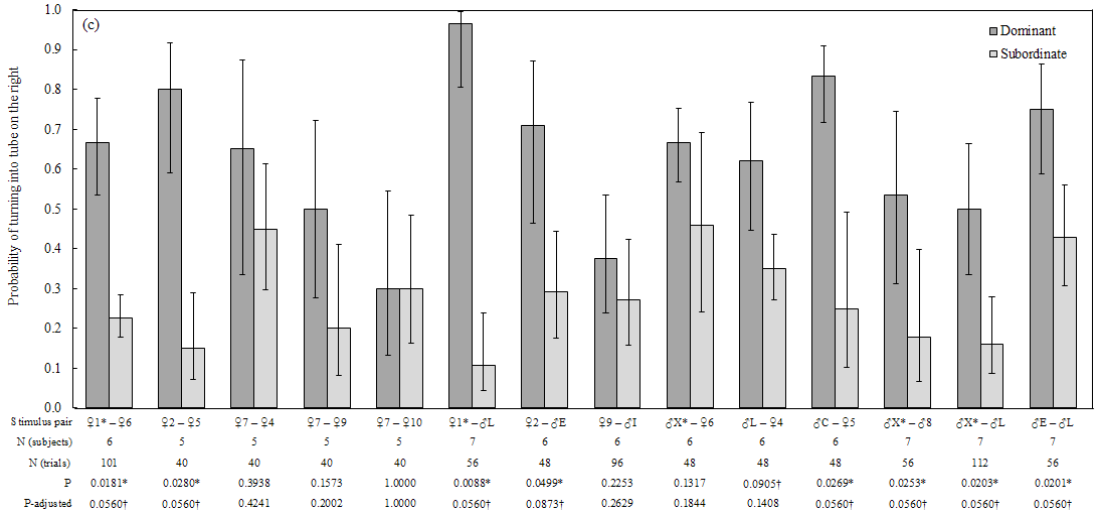
Figure 3.7. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **non-breeder subjects** of (a) both sexes, (b) males, or (c) females. Separate GEE logistic regression models were used for each colony while pooling across multiple stimulus pairs of odor donors (colony 9300: 14 stimulus pairs; colony 1200B: 11 stimulus pairs; and colony 1200D: 10 stimulus pairs). Due to a very small sample size, male and female non-breeders of 1200D were not analyzed separately. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).



Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	11	10	10	10	10	11	10	10	11	10	10	11	11	10
N (trials)	187	80	80	80	80	88	80	160	87	80	80	88	176	80
P	0.0014*	0.0065*	0.8474	0.5316	0.7055	0.0017*	0.0326*	0.0833†	0.4985	0.0307*	0.0063*	0.0285*	0.0108*	0.0253*
P-adjusted	0.0119*	0.0228*	0.8474	0.6202	0.7598	0.0119*	0.0507†	0.1166	0.6202	0.0507†	0.0228*	0.0507†	0.0302*	0.0507†



Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	5	5	5	5	5	4	4	4	5	4	4	4	4	3
N (trials)	86	40	40	40	40	32	32	64	39	32	32	32	64	24
P	0.0274*	0.0455*	0.1797	0.1797	0.6547	0.0858†	0.3657	0.2087	0.7451	0.1025	0.1083	0.5271	0.2498	0.6547
P-adjusted	0.3032	0.3032	0.3594	0.3594	0.7051	0.3032	0.5120	0.3652	0.7451	0.3032	0.3032	0.6709	0.3886	0.7051



Stimulus pair	♀1* – ♀6	♀2 – ♀5	♀7 – ♀4	♀7 – ♀9	♀7 – ♀10	♀1* – ♂L	♀2 – ♂E	♀9 – ♂I	♂X* – ♀6	♂L – ♀4	♂C – ♀5	♂X* – ♂8	♂X* – ♂L	♂E – ♂L
N (subjects)	6	5	5	5	5	7	6	6	6	6	6	7	7	7
N (trials)	101	40	40	40	40	56	48	96	48	48	48	56	112	56
P	0.0181*	0.0280*	0.3938	0.1573	1.0000	0.0088*	0.0499*	0.2253	0.1317	0.0905†	0.0269*	0.0253*	0.0203*	0.0201*
P-adjusted	0.0560†	0.0560†	0.4241	0.2002	1.0000	0.0560†	0.0873†	0.2629	0.1844	0.1408	0.0560†	0.0560†	0.0560†	0.0560†

Figure 3.8. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 9300** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **non-breeder subjects** of (a) both sexes, (b) males, or (c) females. Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

For colony 1200B, non-breeder subjects preferred the dominant stimulus odor in all 11 stimulus pairs and for 6/11 (55%) stimulus pairs this preference was statistically significant, while for 2/11 (18%) stimulus pairs this preference was marginally non-significant after controlling for false discovery (Figure 3.9a). For male non-breeders of colony 1200B, in 10/11 (91%) stimulus pairs, the dominant stimulus odor was preferred, and 4/11 were marginally non-significant after controlling for false discovery rate (Figure 3.9b). For female non-breeders of colony 1200B, in all 11 stimulus pairs the dominant stimulus odor was preferred and in 6/11 (55%) stimulus pairs this preference was marginally statistically significant after controlling for false discovery (Figure 3.9c).

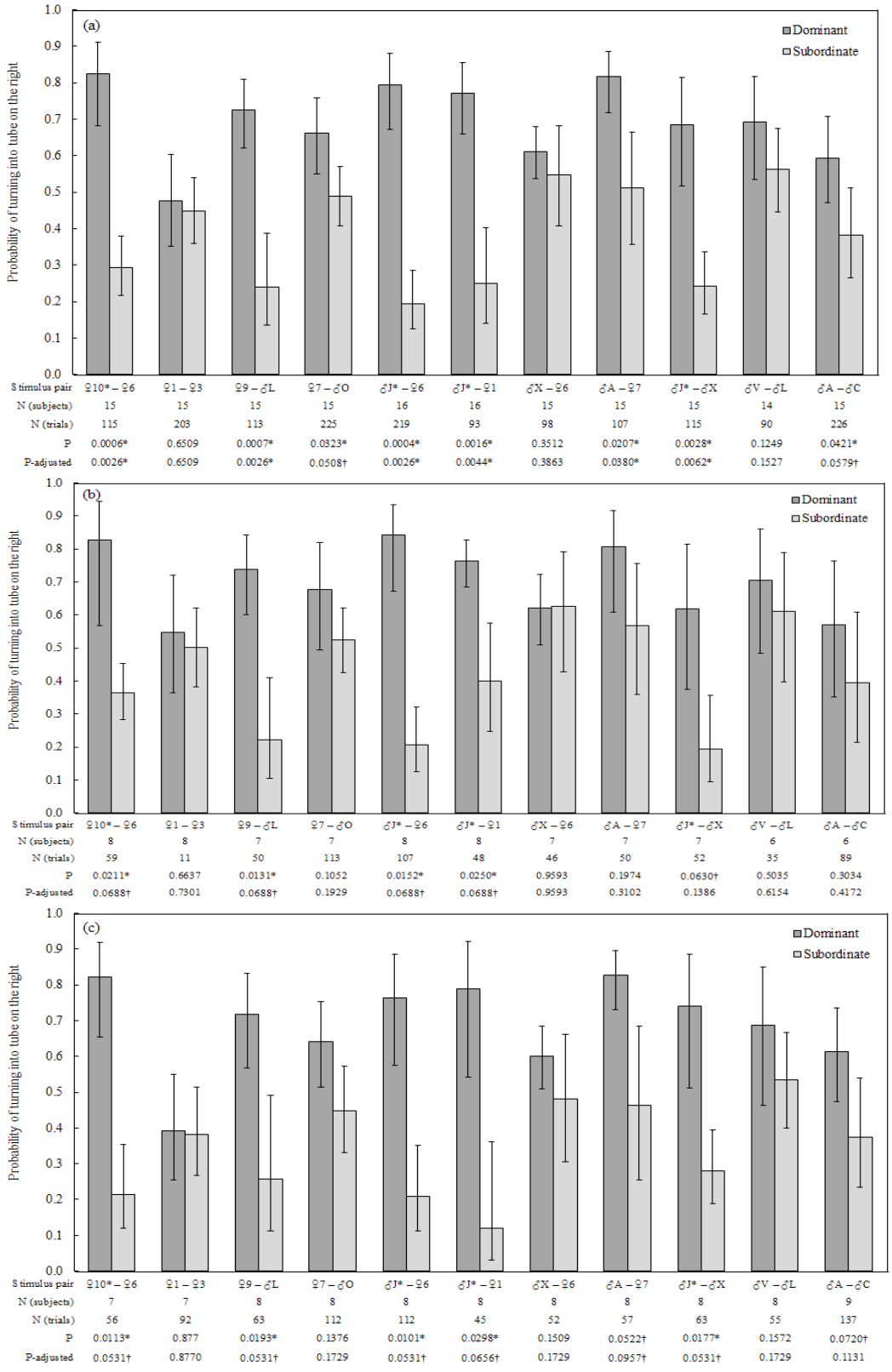

(a)
Dominant
Subordinate
Probability of turning into tube on the right
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Stimulus pair ♀10* – ♀6 ♀1 – ♀3 ♀9 – ♂L ♀7 – ♂O ♂J* – ♀6 ♂J* – ♀1 ♂X – ♀6 ♂A – ♀7 ♂J* – ♂X ♂V – ♂L ♂A – ♂C
N (subjects) 15 15 15 15 16 16 15 15 15 14 15
N (trials) 115 203 113 225 219 93 98 107 115 90 226
P 0.0006* 0.6509 0.0007* 0.0323* 0.0004* 0.0016* 0.3512 0.0207* 0.0028* 0.1249 0.0421*
P-adjusted 0.0026* 0.6509 0.0026* 0.0508† 0.0026* 0.0044* 0.3863 0.0380* 0.0062* 0.1527 0.0579†
(b)
Dominant
Subordinate
Probability of turning into tube on the right
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Stimulus pair ♀10* – ♀6 ♀1 – ♀3 ♀9 – ♂L ♀7 – ♂O ♂J* – ♀6 ♂J* – ♀1 ♂X – ♀6 ♂A – ♀7 ♂J* – ♂X ♂V – ♂L ♂A – ♂C
N (subjects) 8 8 7 7 8 8 7 7 7 6 6
N (trials) 59 11 50 113 107 48 46 50 52 35 89
P 0.0211* 0.6637 0.0131* 0.1052 0.0152* 0.0250* 0.9593 0.1974 0.0630† 0.5035 0.3034
P-adjusted 0.0688† 0.7301 0.0688† 0.1929 0.0688† 0.0688† 0.9593 0.3102 0.1386 0.6154 0.4172
(c)
Dominant
Subordinate
Probability of turning into tube on the right
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Stimulus pair ♀10* – ♀6 ♀1 – ♀3 ♀9 – ♂L ♀7 – ♂O ♂J* – ♀6 ♂J* – ♀1 ♂X – ♀6 ♂A – ♀7 ♂J* – ♂X ♂V – ♂L ♂A – ♂C
N (subjects) 7 7 8 8 8 8 8 8 8 8 9
N (trials) 56 92 63 112 112 45 52 57 63 55 137
P 0.0113* 0.877 0.0193* 0.1376 0.0101* 0.0298* 0.1509 0.0522† 0.0177* 0.1572 0.0720†
P-adjusted 0.0531† 0.8770 0.0531† 0.1729 0.0531† 0.0656† 0.1729 0.0957† 0.0531† 0.1729 0.1131

Figure 3.9. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 1200B** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **non-breeder subjects** of (a) both sexes, (b) males, or (c) females. Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

Breeders were analyzed separately from non-breeders and pooled across the three colonies and stimulus pairs. Queens and breeder males showed a preference for the dominant stimulus odor, but this preference was only marginally non-significant when the sexes were combined (Figure 3.10). A secondary breeder male of colony 9300 was excluded from analyses shown in Figure 3.10 because this male died early in the study before much data was collected, but results were very similar in analyses that included this male (data not shown).

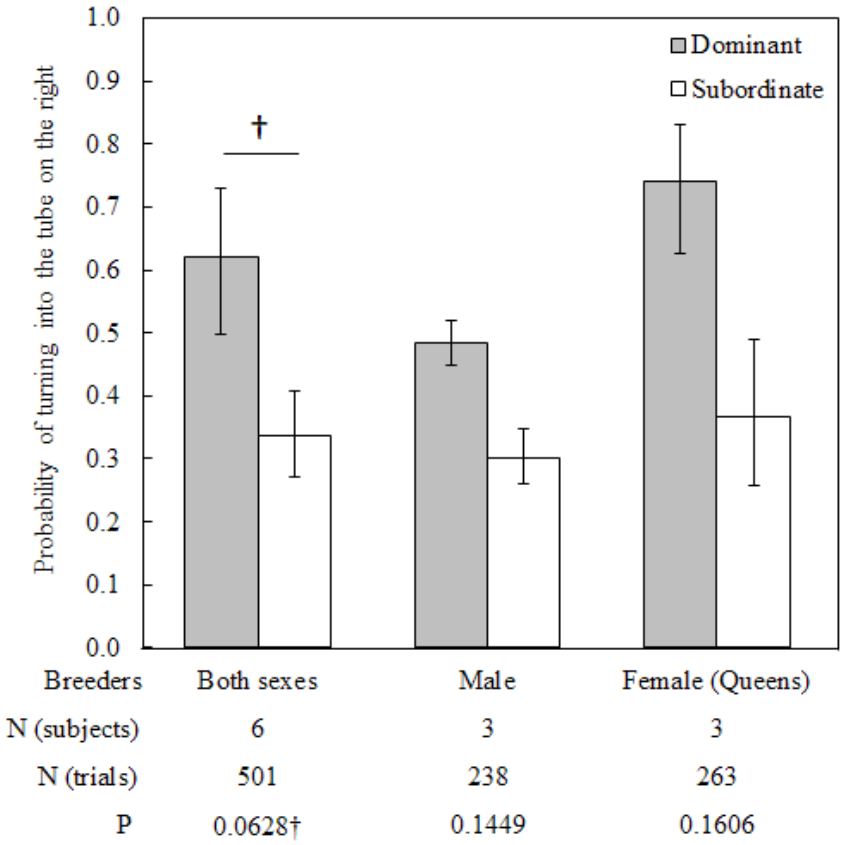
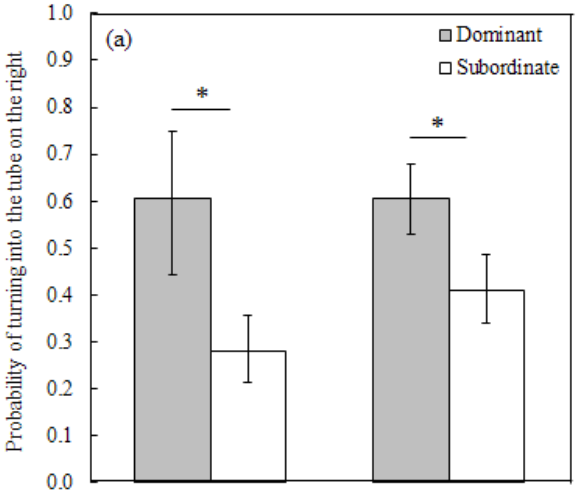


Figure 3.10. Probability of turning into the right arm of a T-choice apparatus by **breeder male and breeder female (queen)** naked mole-rats (*Heterocephalus glaber*) of three colonies as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members. Separate GEE logistic regression models were used for each sex and their combination while pooling across multiple stimulus pairs of odor donors and three colonies, each with one queen and one breeder male (both sexes: colony 9300: 14 stimulus pairs; colony 1200B: 11 stimulus pairs; colony 1200D: 9 stimulus pairs; male breeders: colony 9300: 11 stimulus pairs; colony 1200B: 8 stimulus pairs; colony 1200D: 6 stimulus pairs; queens: colony 9300: 12 stimulus pairs; colony 1200B: 10 stimulus pairs; colony 1200D: 6 stimulus pairs). Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) and (†) indicates a marginally nonsignificant trend.

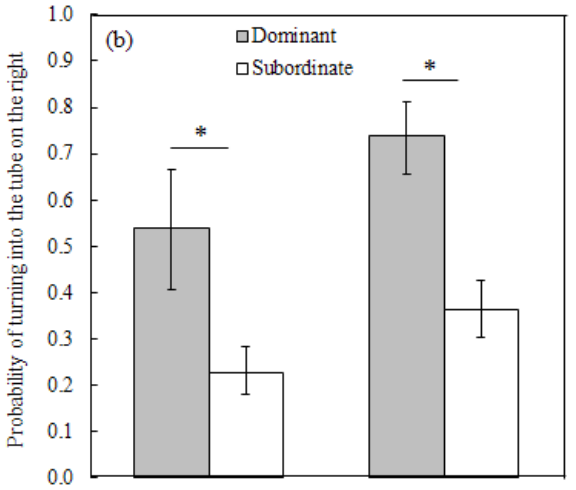
Does dominance-based discrimination depend on subject's absolute dominance rank?

No. Turning preferences were reanalyzed separately using subjects that ranked in the most dominant and most subordinate one-thirds (approximately) of their colonies, and the overall pattern of results for both of these analyses was consistent with each other and the analyses of all subjects, i.e., both the most dominant subjects and the most subordinate subjects showed a preference for the odor of the more dominant stimulus.

For the most dominant subjects, when the T-choice data were analyzed separately for each of the two larger colonies while pooling across all stimulus pairs tested, the probability of turning into dominant stimulus arm was significantly greater for both colony 9300 (logistic regression: $\chi^2_1 = 4.07$, $P_{adj} = 0.044$; Figure 3.11a) and colony 1200B (logistic regression: $\chi^2_1 = 5.62$, $P_{adj} = 0.036$; Figure 3.11a). Similarly, for the most subordinate subjects, the dominant stimulus odor was preferred for both colony 9300 (logistic regression: $\chi^2_1 = 4.05$, $P_{adj} = 0.044$; Figure 3.11b) and colony 1200B (logistic regression: $\chi^2_1 = 5.33$, $P_{adj} = 0.042$; Figure 3.11b).



Colony	9300	1200B
N (subjects)	5	6
N (trials)	579	511
P	0.0437*	0.0177*
P-adjusted	0.0437*	0.0354*



Colony	9300	1200B
N (subjects)	5	6
N (trials)	581	639
P	0.0442*	0.0210*
P-adjusted	0.0442*	0.0420*

Figure 3.11. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **two colonies** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects that ranked** in the (a) **most dominant**, or (b) **most subordinate** approximately **one-third of the colony** (colony 9300: 36%; colony 1200B: 32%). Separate GEE logistic regression models were used for each colony while pooling across multiple stimulus pairs of odor donors (colony 9300: 14 stimulus pairs; colony 1200B: 11 stimulus pairs). Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

For colony 9300, the most dominant subjects preferred the dominant stimulus odor in all 14 stimulus pairs, but none of these differences were statistically significant after controlling for false discovery (Figure 3.12a). For the most subordinate subjects of colony 9300, in 12/14 (86%) stimulus pairs the dominant stimulus odor was preferred, but no difference was statistically significant after controlling for false discovery rate in the 12 stimulus pairs that were able to be analyzed (Figure 3.12b).

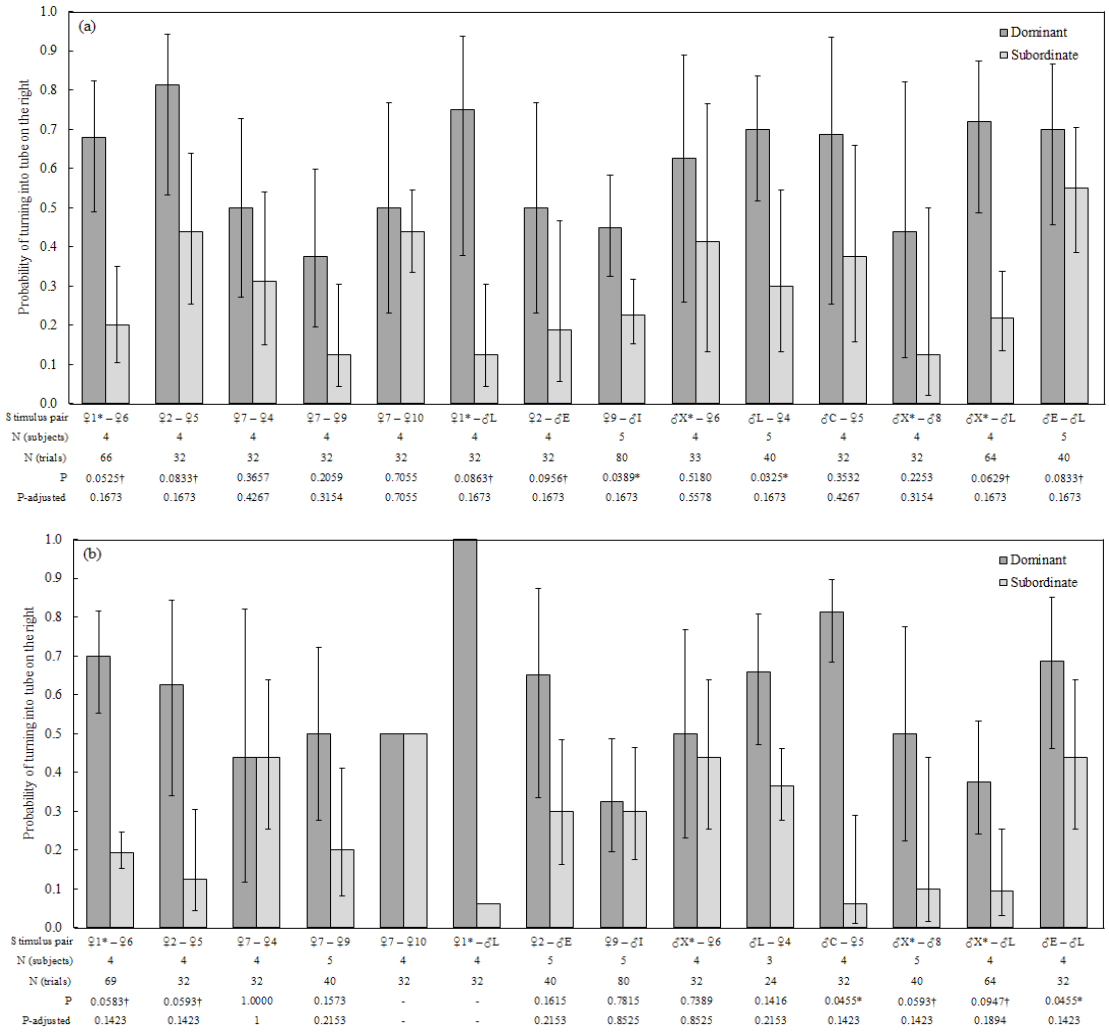


Figure 3.12. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 9300** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects that ranked in the** (a) **dominant**, or (b) **subordinate** 36% of the colony (up to 5/14 subjects). Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Analyses could not be conducted on stimulus pair 7-10 because turning was exactly even between the dominant and subordinate stimulus arms and stimulus pair 1-L because all but one turn was into the dominant stimulus arm. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

For colony 1200B, the most dominant subjects preferred the dominant stimulus odor in 7/11 (64%), but none of these differences were statistically significant after controlling for false discovery (Figure 3.13a). For the most subordinate subjects of colony 1200B, in all 11 stimulus pairs was the dominant odor preferred, while no difference was statistically significant, preference for the dominant odor was marginally statistically non-significant in 6/11 (55%) stimulus pairs after controlling for false discovery (Figure 3.13b).

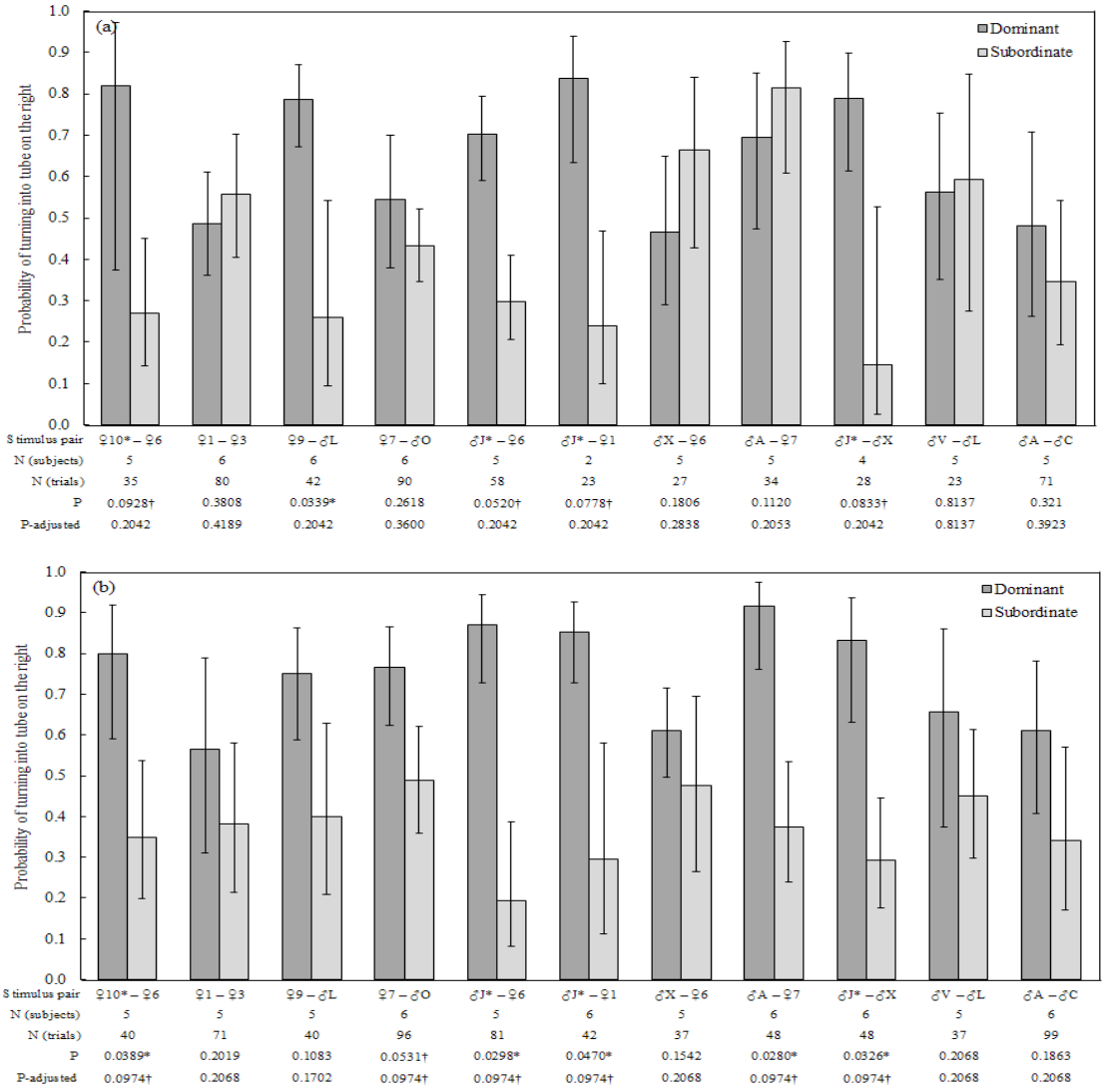


Figure 3.13. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 1200B** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects that ranked in the** (a) **dominant**, or (b) **subordinate** 32% of the colony (up to 6/19 subjects). Separate GEE logistic regression models were used for each stimulus pair tested. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

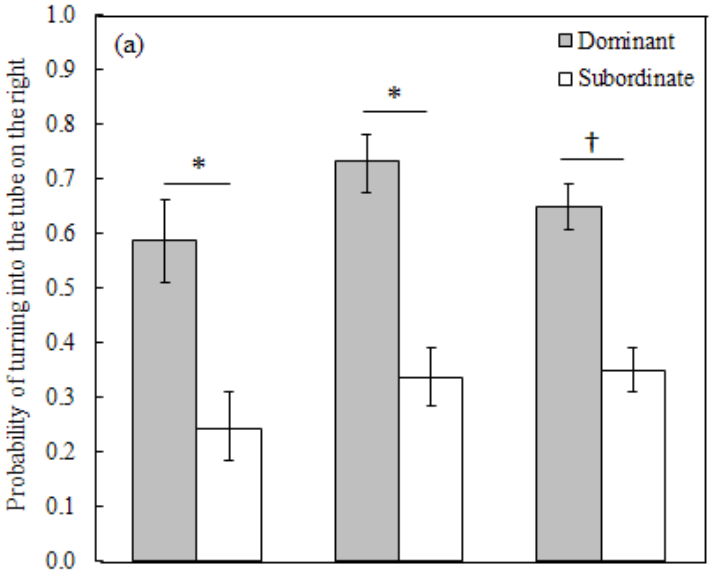
Does dominance-based discrimination depend on subject's dominance rank relative to stimulus odor donors?

No. To see if a subject's discrimination between stimulus odors based on dominance of the odor donor was influenced by the subject's relative dominance rank to those odor donors, turning preferences were reanalyzed separately using subjects with a dominance rank that was between and not between the dominance ranks of the two stimulus odor donors. The overall pattern of results for these two categories of subjects was consistent with each other and the analyses of all subjects combined, i.e., a preference for the odor of the more dominant stimulus.

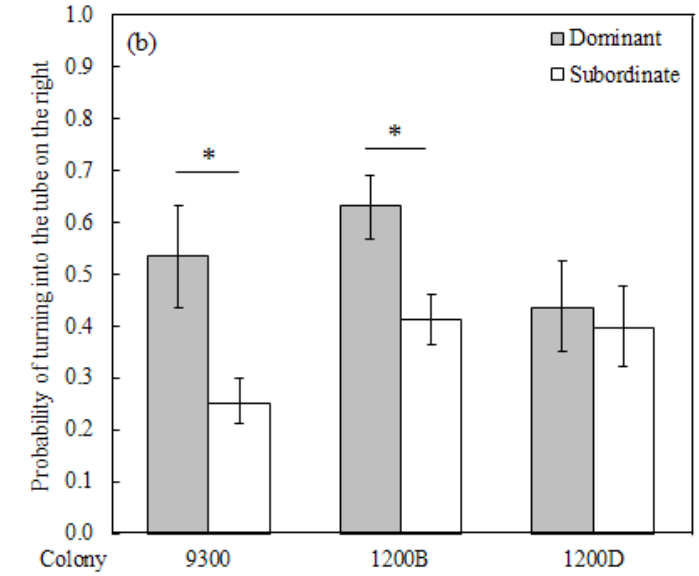
For the subjects with dominance ranks that were between the ranks of the stimulus odor donors, when the T-choice data were analyzed separately for each colony while pooling across all stimulus pairs tested, the probability of turning into dominant stimulus arm was significantly greater for both colony 9300 (logistic regression: $\chi^2_1 = 9.20$, $P_{adj} = 0.004$; Figure 3.14a) and colony 1200B (logistic regression: $\chi^2_1 = 13.07$, $P_{adj} = 0.001$; Figure 3.14a), while this preference was marginally non-significant for colony 1200D (logistic regression: $\chi^2_1 = 2.88$, $P_{adj} = 0.090$; Figure 3.14a).

Similarly, for the subjects with dominance ranks that were not between the ranks of the stimulus odor donors, i.e., subjects with dominance ranks that were either lower or higher than both stimulus odor donors, the dominant stimulus odor was preferred for both colony 9300 (logistic regression: $\chi^2_1 = 6.98$, $P_{adj} = 0.012$; Figure 3.14b) and colony 1200B (logistic regression: $\chi^2_1 = 11.84$, $P_{adj} = 0.002$; Figure 3.14b),

while this preference was not significant for colony 1200D (logistic regression: $\chi^2_1 = 0.31$, $P_{adj} = 0.578$; Figure 3.14b).



Colony	9300	1200B	1200D
N (subjects)	12	17	3
N (trials)	827	779	80
P	0.0024*	0.0003*	0.0897†
P-adjusted	0.0036*	0.0009*	0.0897†



Colony	9300	1200B	1200D
N (subjects)	14	19	5
N (trials)	817	1012	160
P	0.0082*	0.0006*	0.5775
P-adjusted	0.0123*	0.0018*	0.5775

Figure 3.14. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **three colonies** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects with a dominance rank** that was (a) **between**, or (b) **not between the dominance ranks of the two stimulus odor donors**. Separate GEE logistic regression models were used for each colony while pooling across multiple stimulus pairs of odor donors (between: colony 9300: 13 stimulus pairs; colony 1200B: 10 stimulus pairs; colony 1200D; 6 stimulus pairs; not between: colony 9300: 13 stimulus pairs; colony 1200B: 10 stimulus pairs; colony 1200D; 9 stimulus pairs). Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

For colony 9300, 10 stimulus pairs could be analyzed with subjects that ranked between the stimulus odor donors and in all 10, the dominant odor was preferred, and this preference was significant in 2/10 (20%) pairs and marginally non-significant in 2/10 pairs (20%) after controlling for false discovery (Figure 3.15a). For the three stimulus pairs that had any subjects but could not be analyzed, the dominant was preferred in two and turning was exactly even in the third (Figure 3.15a). Eleven stimulus pairs could be analyzed with subjects that ranked not between the stimulus odor donors and in 10/11 (91%) the dominant odor was preferred, and although no difference was significant, 6/11 (55%) were marginally non-significant after controlling for false discovery (Figure 3.15b). For the two additional stimulus pairs that could not be analyzed due to too few subjects and a lack of variation, all turns were towards the dominant odor (Figure 3.15b).

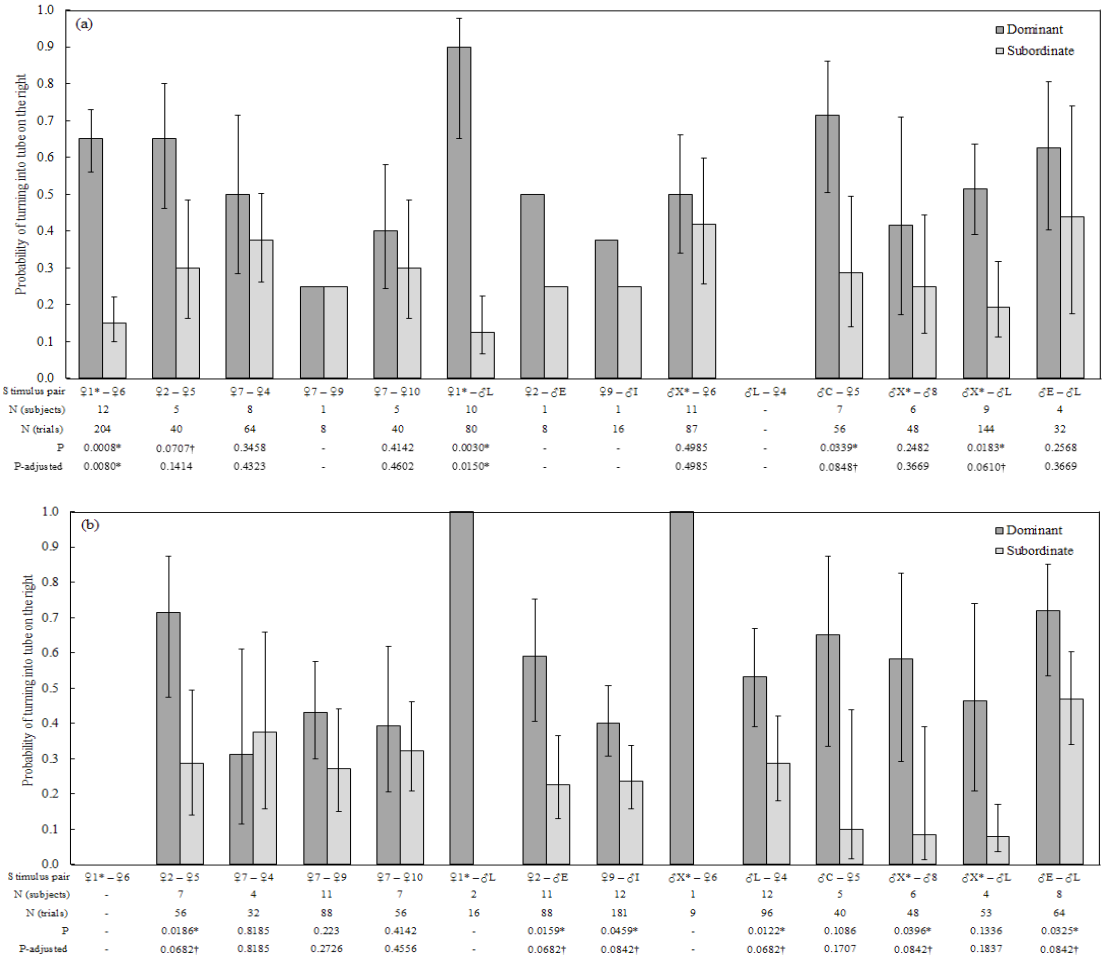


Figure 3.15. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 9300** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects with dominance ranks that were** (a) **between** or (b) **not between the dominance rank of the two stimulus odor donors**. Separate GEE logistic regression models were used for each stimulus pair tested. Analyses could not be conducted for: "between" stimulus pairs 7-9, 2-E, and 9-I because only one subject for each pair was ranked between odor donors, and L-4 because no subjects were ranked between the odor donors; "not between" stimulus pairs: 1-6 because no subjects were ranked not between the odor donors, and 1-L and X-6 because of too few subjects. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

For colony 1200B, eight stimulus pairs could be analyzed with subjects that ranked between the stimulus odor donors and in all 8, the dominant odor was preferred, and this preference was significant in 4/8 (50%) pairs and marginally non-significant in 1/8 pairs (13%) after controlling for false discovery (Figure 3.16a). For the two stimulus pairs that had any subjects but could not be analyzed, the dominant was preferred in one of the two (Figure 3.16a). Nine stimulus pairs could be analyzed with subjects that ranked not between the stimulus odor donors and in 8/9 (89%) the dominant odor was preferred, and in 1/9 (11%) this preference was statistically significant and in 4/9 (44%) this preference was marginally non-significant (Figure 3.16b). For the one stimulus pair that had only one potential subject and could not be analyzed, the sole subject, breeder male "J," preferred subordinate female "6" over dominant male "X." Interestingly, the most common casual observations of aggression in colony 1200B were shoves by male "J" directed towards female "6."

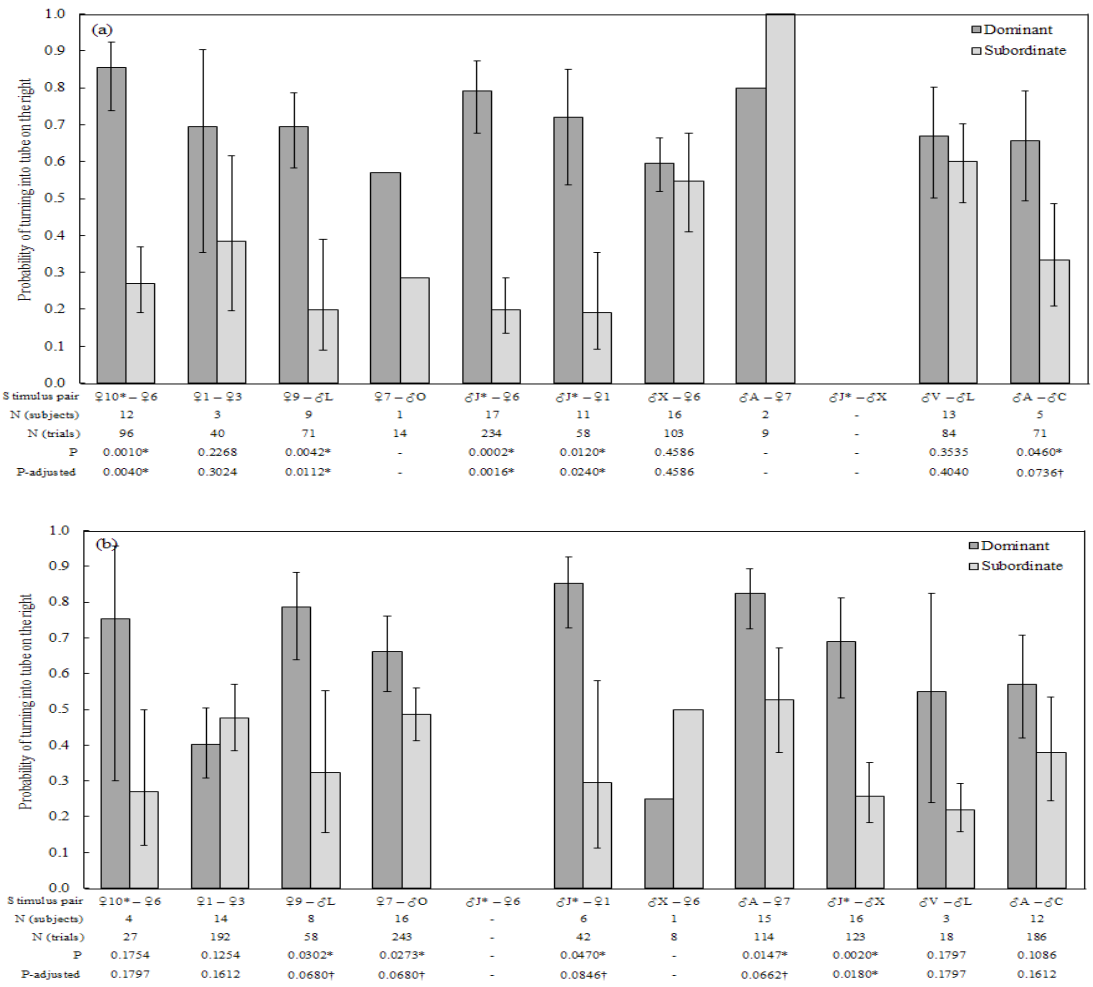


Figure 3.16. Probability of turning into the right arm of a T-choice apparatus by naked mole-rats (*Heterocephalus glaber*) of **colony 1200B** as a function of whether the arm contained the odor of a more dominant or more subordinate of two fellow colony members analyzed using **subjects with dominance ranks that were** (a) **between** or (b) **not between the dominance rank of the two stimulus odor donors**. Separate GEE logistic regression models were used for each stimulus pair tested. Analyses could not be conducted for: "between" stimulus pairs 7-O, and A-7 because of too few subjects, and J-X because no subjects were ranked between the odor donors; "not between" stimulus pairs: J-6 because no subjects were ranked not between the odor donors, and X-6 because of too few subjects. Bars represent mean probabilities with 95% confidence intervals converted from logit LS-means. Stimulus pairs are labeled with the dominant odor donor first; breeders are denoted with (*). A "trial" is one choice by an individual; repeated measures for each subject were controlled for in the models. Statistical significance is reported unadjusted (P) or adjusted for multiple hypothesis testing using false discovery rate (P-adjusted); statistical significance is denoted with (*) and marginally nonsignificant trends with (†).

In addition to subject characteristics, dominance-based odor discrimination may be influenced by the characteristics of the odor donors, aside from their dominance ranks, which generates the following questions. Answers to these questions were obtained by looking at the results of individual stimulus pairs. Table 3.2 lists stimulus pairs of odor donors organized by odor donor characteristics for which dominance-based discrimination by subjects was statistically significant and lists the figures where the results of each analysis can be found.

Table 3.2. Stimulus pairs of odor donors organized by odor donor characteristics for which dominance-based discrimination by subjects was statistically significant in two colonies of naked mole-rats (*Heterocephalus glaber*) and the figures where the results of each analysis can be found when all subjects were included in analyses. For each stimulus pair, the odor of the dominant donor was significantly preferred before and after controlling for false discovery rate.

Stimulus odor donor characteristics	Dominant stimulus odor donor	Subordinate stimulus odor donor	Colony	Figure	Stimulus pairs with dominance-based discrimination
sex, breeding status	male non-breeder	male non-breeder	9300	3.4a	♂E–♂L
			1200B	3.5a	♂A–♂C
	male breeder	male non-breeder	9300	3.4a	♂X–♂8, ♂X–♂L
			1200B	3.5a	♂J–♂X
	female non-breeder	female non-breeder	9300	3.4a	♀2–♀5
			1200B	3.5a	
	female breeder	female non-breeder	9300	3.4a	♀1–♀6
			1200B	3.5a	♀10–♀6
	male non-breeder	female non-breeder	9300	3.4a	♂L–♀4, ♂C–♀5
			1200B	3.5a	♂A–♀7
	male breeder	female non-breeder	9300	3.4a	♂X–♀6
			1200B	3.5a	♂J–♀6, ♂J–♀1
	female non-breeder	male non-breeder	9300	3.4a	♀2–♂E, ♀9–♂I
			1200B	3.5a	♀9–♂L, ♀7–♂O
	female breeder	male non-breeder	9300	3.4a	♀1–♂L
			1200B	3.5a	
body weight	lighter	heavier	9300	3.4a	
			1200B	3.5a	♂J–♂X, ♀9–♂L
	similar	similar	9300	3.4a	♀9–♂I, ♂L–♀4
			1200B	3.5a	♂J–♀1, ♂A–♀7, ♂A–♂C

Does dominance-based discrimination depend on stimulus sex or stimulus breeding status?

No, but discrimination was mostly stronger when the dominant odor donor was a breeder. To see if the sex or breeding status of the stimulus odor donor influences turning preference of subjects, results of the different stimulus pairs were compared. Four stimulus pair sex combinations were possible: male dominant to male, female dominant to female, male dominant to female, and female dominant to male. For each of these sex combinations, the dominant individual could be a breeder or non-breeder, leading to a total of eight types of stimulus pairs. Consistent with the overall finding that the dominant stimulus odor is preferred, there are examples of a significant preference after controlling for false discovery for the dominant stimulus odor for each of these eight stimulus pair types across the 25 stimulus pairs tested of the two larger colonies (summarized below; Figures 3.4a & 3.5a).

Examples of a significant preference for the dominant stimulus odor existed for all types of same-sex stimulus pairs. The dominant odor was preferred when the stimulus odor donors were both non-breeding females in colony 9300 for stimulus pair ♀2–♀5 (Figure 3.4a). The dominant queen's odor was preferred over a subordinate non-breeder female in colony 9300 for stimulus pair ♀1–♀6 (Figure 3.4a) and in colony 1200B for stimulus pair ♀10–♀6 (Figure 3.5a). The dominant odor was preferred when the stimulus odor donors were both non-breeding males in colony 9300 for stimulus pair ♂E–♂L (Figure 3.4a) and in colony 1200B for stimulus pair ♂A–♂C (Figure 3.5a). The dominant breeder male's odor was preferred over a

subordinate non-breeder male in colony 9300 for stimulus pairs ♂X–♂8 and ♂X–♂L (Figure 3.4a) and in colony 1200B for stimulus pair ♂J–♂X (Figure 3.5a).

Examples of a significant preference for the dominant stimulus odor existed for all types of opposite-sex stimulus pairs. The dominant odor was preferred when the dominant stimulus was a non-breeder female and the subordinate stimulus was non-breeder male in colony 9300 for stimulus pairs ♀2–♂E and ♀9–♂I (Figure 3.4a) and in colony 1200B for stimulus pairs ♀9–♂L and ♀7–♂O (Figure 3.5a). The dominant queen's odor was preferred when the subordinate was a non-breeder male in colony 9300 for stimulus pair ♀1–♂L (Figure 3.4a). The dominant odor was preferred when the dominant stimulus was a non-breeder male and the subordinate stimulus was a non-breeder female in colony 9300 for stimulus pairs ♂L–♀4 and ♂C–♀5 (Figure 3.4a) and in colony 1200B for stimulus pair ♂A–♀7 (Figure 3.5a). The dominant breeder male's odor was preferred when the subordinate was a non-breeder female in colony 9300 for stimulus pair ♂X–♀6 (Figure 3.4a) and in colony 1200B for stimulus pairs ♂J–♀6 and ♂J–♀1 (Figure 3.5a).

Furthermore, the preference for the dominant stimulus remained significant in most of the stimulus pairs mentioned above when they were reanalyzed using only female subjects, with a significant preference for the dominant stimulus being shown in at least one stimulus pair for each of the eight types of stimulus pairs (compare Figure 3.4a with 3.4c and Figure 3.5a with 3.5b for those stimulus pairs noted above).

When only male subjects were analyzed, no preference for the dominant stimulus was statistically significant after controlling for false discovery, likely due to

the lower statistical power of fewer male subjects than female subjects. Nevertheless, there are examples of a marginally non-significant preference for the dominant odor after controlling for false discovery or a significant preference prior to controlling for false discovery for 5/8 stimulus pair types (two non-breeder females, queen dominant to non-breeder female, queen dominant to non-breeder male, non-breeder female dominant to non-breeder male, and breeder male dominant to non-breeder female; compare Figure 3.4a with 3.4b and Figure 3.5a with 3.5b). For stimulus pairs in which a non-breeder male was dominant to a non-breeder female, there is an example of male subjects showing a marginally non-significant preference for the dominant stimulus prior to controlling for false discovery (colony 9300, stimulus pair ♂L–♀4; Figure 3.4b). For stimulus pairs in which the breeder male was dominant to a non-breeder male, there is an example of male subjects showing a marginally non-significant preference for the dominant stimulus prior to controlling for false discovery (colony 1200B, stimulus pair ♂J–♂X; Figure 3.5b). Lastly, for stimulus pairs with two non-breeder males, there are only examples of non-significant preferences for the dominant stimulus odor (colony 9300, stimulus pair ♂E–♂L; colony 1200B, stimulus pairs ♂V–♂L and ♂A–♂C; Figures 3.4b & 3.5b).

Consistently across analyses, the preference for the odor of the more dominant of two colony members was the strongest when the dominant odor donor was a breeder, especially when the breeder was the queen (Figures 3.4, 3.5, 3.6, 3.8, 3.9, 3.12, 3.13, 3.15, & 3.16).

Is dominance-based discrimination actually weight-based discrimination?

No. Despite a strong correlation between dominance rank and body weight such that heavier individuals are more dominant (Figure 3.1), the preference for the dominant stimulus odor extended to stimulus pairs in which the subordinate stimulus odor donor was heavier than the dominant stimulus odor donor and the dominant stimulus was preferred in stimulus pairs in which the weight difference between the stimulus odor donors was very minimal.

In colony 1200B, non-breeder male "X" was approximately 51% heavier than breeder male "J" throughout the course of the study, yet there was a significant preference for the dominant breeder male "J" when male and female subjects were analyzed together (Figure 3.5a) and when female subjects were analyzed separately (Figure 3.5c). Male subjects also showed a preference for the dominant less heavy breeder male "J," but this preference was only marginally non-significant prior to controlling for false discovery (Figure 3.5b).

Preference for the odor of a dominant stimulus over a heavier subordinate stimulus was also seen in a stimulus pair with non-breeders of opposite sex. In colony 1200B, non-breeder male "L" was 19% heavier than dominant non-breeder female "9" throughout the study (Figure 3.1b), yet there was a significant preference for the dominant female "9" when male and female subjects were analyzed together (Figure 3.5a) and when female subjects were analyzed separately (Figure 3.5c). Male subjects also showed a preference for the dominant less heavy female "9" but this preference was marginally non-significant after controlling for false discovery (Figure 3.5b).

In five stimulus pairs across the two larger colonies, the dominant stimulus odor (which was also the odor from the heavier stimulus in half of the pairs) was significantly preferred despite one stimulus being only 0-7% heavier than the other (Colony 9300: stimulus pairs ♀9–♂I and ♂L–♀4; Figure 3.4a; Colony 1200B: stimulus pairs ♂J–♀1, ♂A–♀7, and ♂A–♂C 3.5a).

Is the preference for the dominant stimulus odor stronger when the stimulus pair rank difference is greater?

No, not if stimulus pairs that include a breeder are excluded from analyses. The probability of choosing to enter the T-choice arm with the dominant stimulus odor was significantly influenced by the rank difference between the two stimulus odor donors for the two larger colonies, colony 9300 (logistic regression: $\chi^2_1 = 8.56$, $N = 14$ stimulus pairs, $N = 15$ subjects, $N = 1658$ trials, $P = 0.003$) and colony 1200B (logistic regression: $\chi^2_1 = 10.36$, $N = 11$ stimulus pairs, $N = 19$ subjects, $N = 1791$ trials, $P = 0.001$), but not the small colony, colony 1200D (logistic regression: $\chi^2_1 = 2.01$, $N = 10$ stimulus pairs, $N = 5$ subjects, $N = 240$ trials, $P = 0.156$). However, the significant results of colony 9300 and colony 1200B were no longer significant when analyses excluded stimulus pairs that included queens or male breeders or excluded only stimulus pairs that included queens (data not shown).

DISCUSSION

Summary of Results

Overall, the results strongly support the hypothesis that naked mole-rats can discriminate between the odors of fellow colony members based on the dominance status of odor donors. Furthermore, the overall pattern of results revealed a preference for the odor of the dominant stimulus donor over the odor of the subordinate stimulus donor, as measured by entering the arm of a T-choice apparatus. This overall pattern of preference for the dominant stimulus odor was consistent across every category of subject analyzed and every category of stimulus odor donor analyzed. Finer scale analysis of particular stimulus pairs tested within a single colony did not always reveal a statistically significant preference for the dominant odor, particularly when samples sizes were small, but for the majority of stimulus pairs, the dominant odor was approached more often, and for no stimulus pair across the entire study was there a statistically significant preference for the subordinate odor.

Non-breeder and breeder naked mole-rats of both sexes can discriminate between odors based on the dominance of odor donors and both sexes prefer the dominant odor. The pattern of preference for the dominant stimulus odor was consistent across male and female subjects and whether or not breeders (queens and breeding males) were excluded from analyses. Given that only three colonies were tested and each colony only had one queen and one breeder male, the sample size for breeders as subjects was small. Nonetheless, queens and breeder males more often approached the dominant odor and, when the six breeders were analyzed together,

there was a non-significant trend to approach the dominant odor. In the larger context of the study, it is very likely that this observed preference for dominant odor is biologically meaningful, but further study is needed with a larger sample size of breeders to obtain conventional statistical significance and conclude with greater confidence.

Both the most dominant and the most subordinate subjects within a colony showed a preference for the dominant stimulus odor. In other words, preference for the dominant odor was found when analyses were restricted to individuals that ranked in either the top or bottom (approximately) one-third of the colony's dominance hierarchy. These results suggest that individuals of all dominance ranks are motivated to approach the odor of dominant individuals, whether or not they have congruent reasons for doing so (see below). Furthermore, the preference for the dominant odor was not dependent on the subject's dominance rank relative to the dominance rank of either stimulus odor donor. That is, subjects did not have to be presented with odor from a donor that was dominant to the subject and a donor that was subordinate to the subject in order for the dominant odor to be preferred. Subjects with dominance ranks that were not between the ranks of either odor donor (were either above both odor donors or below both odor donors) still showed a preference for the dominant odor. These results demonstrate that an individual's ability to discriminate between fellow colony members is not limited to their own dominance relationships relative to others and that individuals do not merely have a preference for approaching odors of fellow colony members that are more dominant than themselves.

Preferences for the dominant stimulus odor were found whether both odor donors were male, both were female, the dominant was female and the subordinate was male, or the dominant was male and the subordinate was female. Preferences for the dominant odor were also found whether or not the dominant was a breeder in each of these four types of stimulus pairs. The overall patterns were also consistent when subjects were analyzed separately by sex. Clearly, the ability to discriminate between the odors of fellow colony members based on dominance is not restricted within one sex, towards only the opposite sex, or based solely on breeding status. This is perhaps unsurprising when considering the fact that a colony's linear dominance hierarchy is interspersed with males and females from the most dominant breeders and non-breeders to the most subordinate non-breeders (Clarke & Faulkes, 1997; Schieffelin & Sherman, 1995; this study). Furthermore, consistently across analyses, the preference for the dominant odor was strongest when the dominant odor donor was a breeder, particularly when the breeder was the queen. This may indicate that discrimination between breeders and non-breeders is easier to do, perhaps due to additional chemical cues that breeders exude due to being in a reproductive state or due to the fact that breeder odor donors tended to urinate and defecate when isolated in a stimulus arm. Although efforts were made to prevent urine and feces excreted by stimulus odor donors from being present in stimulus arms, it is possible that trace amounts, enough to be detected by subjects, were transferred to the arms by the stimulus odor donors. The stronger preference for the dominant odor when the dominant odor donor was a breeder may also indicate that individuals are more strongly motivated to approach the

odors of breeders than non-breeders or are motivated to approach the odor of urine and/or feces more than other odors.

The preference for odor from a dominant stimulus donor persisted despite the potential confound between odor donor dominance rank and body weight. In all three colonies, body weight was significantly correlated with dominance rank, determined via agonistic interactions, including passing behavior, such that more dominant individuals tended to be heavier. This finding is consistent with other studies in which dominance was determined by passing behavior (Clarke & Faulkes, 1997, 1998; Toor, Clement, Carlson, & Holmes, 2015) or tugging contests for food (Schieffelin & Sherman, 1995). Nevertheless, there were a couple of stimulus pairs tested in which the subordinate odor donor was heavier than the dominant odor donor and the odor from the dominant donor was still preferred. In one of these stimulus pairs, the dominant odor donor was the colony's breeder male and most dominant individual. After achieving breeder status, breeder males many lose body weight over their lifetime and will appear emaciated (Jarvis, O'Riain, & McDaid, 1991), as did this breeder male. This male showed no signs of illness and maintained an emaciated appearance for over 10 years. In the other stimulus pair, the dominant odor donor was not a breeder, demonstrating that preference for the lighter dominant cannot be explained by breeding status. Furthermore, when the difference in body weight between the two stimulus odor donors was very minimal, the odor from the dominant donor was still preferred. These results suggest that the ability to discriminate between the odors of fellow colony members based on dominance is not merely due to a higher

total quantity of all odors being released by larger-bodied individuals.

When the dominance rank difference between the stimulus odor donors was larger, there was a stronger preference for the odor of the dominant donor, but this pattern was no longer significant when stimulus pairs that included a breeder as the dominant donor were excluded. This indicates that the preference for the odor of the dominant stimulus donor is strongest when that dominant odor donor is a breeder. This finding is also discussed below in the context of how odor might be able to convey dominance.

The dominance hierarchies of several other species of group-living mole-rats have been described, but this is the first study to demonstrate the ability for dominance-based discrimination in any of these species (common mole-rat, *Cryptomys hottentotus*, Bennett, 1989); Damaraland mole-rat, *Fukomys damarensis*, Jacobs, Bennett, Jarvis, & Crowe, 1991; Mashona mole-rat, *Fukomys darlingi* ,Gabathuler, Bennett, & Jarvis, 1996; giant Zambian mole-rat, *Fukomys mechowii* ,Wallace & Bennett, 1998; highveld mole-rat, *Cryptomys hottentotus pretoriae*, Moolman, Bennett, & Schoeman, 1998).

Why evolve dominance-based discrimination of odors?

Since social dominance plays significant roles in several aspects of naked mole-rat inclusive fitness, including reproductive division of labor, mate choice, access to food, and maintaining cooperation (see Introduction), natural selection has likely favored the evolution of mechanisms for individuals to discriminate between

fellow colony members based on dominance. The current study provides strong evidence that naked mole-rats have evolved such a mechanism, one that uses odor as the basis for discrimination. Further study is needed to elucidate exactly how naked mole-rats use this ability to help maximize their fitness, but I suggest a few possibilities.

First, recognizing dominants by odor may aide in maintaining reproductive suppression of subordinates. Although there is no apparent primer pheromone released by queens to reproductively suppress subordinates (Faulkes & Abbott, 1993; Smith, Faulkes, & Abbott, 1997), the ability to recognize the presence of odor from more dominant colony members may contribute to reproductive suppression in more subtle ways. Although physical interaction with the queen, likely in the form of shoving, was found to be necessary to reproductively suppress subordinates in two other studies (Faulkes & Abbott, 1993; Smith et al., 1997), the need to shove nearly every other individual in a colony seems like an overwhelming task when you consider that the average colony size in nature is roughly 75 animals with a the largest size reported as 295 (Brett, 1991b). It seems more efficient if a queen can suppress individuals by indirectly communicating their presence to subordinates via odor. Similarly, dominant non-breeder females may communicate their presence via odor to more subordinate non-breeders which may contribute to the subordinate's reproductive suppression and prevent the surbordinate from challenging the more dominant non-breeder for queenhood in the event that the queen dies. By recognizing the presence of more dominant non-breeding females that are more likely to win the competition for

queenhood when the queen dies, a subordinate female can avoid personal injury and death. This would also allow for a smooth transition of power and thus prevent a period of social instability during which several individuals may become injured or die and overall cooperation levels are diminished thereby lowering the inclusive fitness of all colony members. Similarly, non-breeder males may use knowledge of the presence of more dominant males to decide if they will become involved in the fighting that may erupt.

Second, odor may be used to recognize dominant individuals for mate choice. Queens may improve their fitness by using odor to recognize and mate with a male that is dominant to many others (male and female) and can help her keep subordinates suppressed, perhaps via shoving since breeder males do the most shoving after queens (Clarke & Faulkes, 2001; Reeve & Sherman, 1991). Indeed, the dominance rank of a male seems to be an important factor in queen mate choice, as breeder males are chosen among the most dominant in the colony (Clarke & Faulkes, 1998). There is evidence that non-breeder males and non-breeder females may form alliances (Ciszek, 2000; Lacey & Sherman, 1997), so non-breeder males may use odor to decide which non-breeder female challenger to ally with and the non-breeder female challengers may use odor to decide which male supporter to mate with once they achieve queenhood.

Third, odor may be used to recognize the dominance of individuals when competing for access to food. A dominant of either sex may use odor to decide to take away a piece of food from a subordinate of either sex and a subordinate may use odor

to allow this to happen without fighting or wasting energy in a struggle.

Fourth, odor may be used to recognize the dominance of other individuals in order to maintain cooperation and a peaceful colony. For example, a subordinate of either sex may act submissively towards a dominant of either sex to avoid injury from fighting. If all individuals know how they should act towards other members of the colony, there will likely be less fighting and more cooperation.

Fifth, odor may be used to recognize the dominance relationship between two fellow colony members and individuals may use that information to decide on whose behalf to intervene in a fight. Spotted hyenas (*Crocuta crocuta*), for example, recognize the dominance relationships between two other conspecifics and most often intervene on behalf of the dominant (Engh, Siebert, Greenberg, & Holekamp, 2005). An interesting finding of the current study is that the ability to discriminate between odors of two fellow colony members existed even if the subject was either more dominant or more subordinate than the two stimulus odor donors. Aggression will sometimes involve more than two individuals simultaneously (personal observations). It may be that if two fellow colony members are fighting nearby, an individual may smell the dominance difference between them and use that information to decide which of the two others to help.

There are likely many other ways in which naked mole-rats can use the ability to discriminate between odors of fellow colony members based on dominance to help maximize their fitness, but additional study is needed to determine exactly what they are.

Why prefer the odor of the dominant donor?

In addition to demonstrating that naked mole-rats can discriminate between the odors of two fellow colony members based on dominance, the current study revealed an overall pattern of preference for the odor of the dominant odor donor over odor from the subordinate odor donor. The preference is indicated by a higher probability that subjects enter the stimulus arm of a T-choice apparatus containing the odor of the dominant stimulus odor donor. If entering a tube that smells like a specific individual is an indication that the subject is interested in approaching that individual, then there are several possible explanations as to why naked mole-rats prefer to approach the odors of dominants and these explanations may differ depending on the characteristics of the subject or stimulus donors.

Breeders (queens and breeder males) may have chosen to go towards the odor of the dominant donor when that donor was their mate because they prefer to be in the proximity to their mate as part of a bonding mechanism or to facilitate interactions that allow for monitoring reproductive state (Jarvis, 1991). Jarvis (1991) reported that in one colony the dominant breeder male was found in the nest with the queen more often than any other colony member and that when the queen moved from one nest box to another this breeder male would usually follow within 10 minutes. Queens and breeder males are also the colony members that engage in the most mutual ano-genital nuzzling in which each breeder nuzzles their nose on the ano-genital region of the other, likely exchanging informative chemicals and thus perhaps monitoring

reproductive state (Ciszek, 2000; Jarvis, 1991; Lacey et al., 1991). Since non-breeder naked mole-rats are reproductively suppressed (Faulkes & Abbott, 1997), it is unlikely that in the current study non-breeder subjects chose to approach the odor of opposite-sex donors over same-sex donors in order to seek mating opportunities. Moreover, non-breeder preference for odor from the dominant donor was found for stimulus pairs in which both donors were of the same sex, both donors were of the opposite-sex, and the dominant donor was either the same sex or opposite sex.

Queens may prefer to go towards the odor of dominant non-breeder donors in order to monitor and behaviorally reproductively suppress individuals that are more likely to pose a challenge to their reproductive status. Studies suggest that shoving (Lacey et al., 1991) by the queen is necessary for reproductive inhibition of non-breeder naked mole-rats (Faulkes & Abbott, 1993; Smith et al., 1997), and in queen removal experiments fighting breaks out among the most dominant colony members, usually resulting in the next highest ranking female replacing the queen (Clarke & Faulkes, 1997). A preference for approaching the more dominant of two individuals may reflect a prioritization of which individuals to monitor and shove. One might expect that a queen may preferentially approach non-breeder females over non-breeder males because females pose a bigger risk to her reproductive dominance and should thus be shoved more often. However, queens did not shove males and females at different rates (Clarke & Faulkes, 2001; Reeve & Sherman, 1991) and in the current study, queens still preferred to go towards odor of a dominant that was male over a subordinate that was female (data not shown). It may be that queens must monitor and

shove non-breeder males as well as females in order to prevent non-breeders of both sexes from disrupting the status quo of her reproductive dominance, as it may be that non-breeder males sometimes support non-breeder females that are challenging to become queen (Ciszek, 2000; Lacey & Sherman, 1997). Consistent with this possibility is the fact that queens and females challenging to be queen sometimes attack and even kill males during periods of social instability (Ciszek, 2000; Clarke & Faulkes, 1997; Jarvis, 1991; Lacey & Sherman, 1991).

Breeder males may prefer to go towards the odor of dominant non-breeder donors in order to maintain dominance over their potential rivals for the queen's mating interest, as naked mole-rat queens control mating (Jarvis, 1991) and exercise mate choice (Ciszek, 2000; Clarke & Faulkes, 1998, 1999), preferring males among the most dominant of the colony (Clarke & Faulkes, 1998). After queens, breeder males are responsible for the highest amount of shoving, directed towards non-breeders (Clarke & Faulkes, 2001; Reeve & Sherman, 1991). One might expect that a breeder male may preferentially approach non-breeder males over non-breeder females because males pose a greater risk to his reproductive dominance, and thus dominance relationships should be constantly reinforced while prioritizing those most likely to pose a threat -those subordinates that are most dominant. However, in the current study, breeder males preferred to go towards odor of a dominant donor even if that donor was a female and the subordinate donor was a male (data not shown). It may be that a breeder male monitors and shoves non-breeder females that pose a threat to the queen (his mate) because a successful usurper of the queen may not choose to

mate with the same males and may even kill them (Clarke & Faulkes, 1997).

Non-breeders and breeders of both sexes may prefer to go towards the odor of the more dominant of two fellow colony members because gravitating towards the odor of dominants may function to help individuals locate the currently used nest which may change location in their vast tunnel system ("the nest-locating hypothesis"). The naked mole-rat is the only known poikilothermic mammal (Buffenstein & Yahav, 1991), and communal huddling is important for thermoregulation and minimizing evaporative water loss (Withers & Jarvis, 1980; Yahav & Buffenstein, 1991). Naked mole-rats spend a lot of time resting and huddling together in a nest chamber but temporarily disperse to forage, excavate new tunnels, and perform other activities (Lacey et al., 1991). In the wild, (Brett, 1991a) radio-tracked several members of a colony of 87 naked mole-rats and determined that the total span of their tunnel system was over 3 km long. Furthermore, tunnel systems may have multiple nest chambers and colonies will alternate between them. In the colony mapped by Brett (1991a), eleven nest sites were determined by the tracking of several radio-tagged individuals repeatedly to the same location where they simultaneously remained stationary, but usually only one nest was used at any given time and no more than 2-3 were used simultaneously. Since an individual can potentially travel far from the nest site and the location of the nest site may change while an individual is away, natural selection may favor the evolution of a mechanism for efficiently locating the new nest location. Furthermore, since more dominant individuals are heavier (Clarke & Faulkes, 1997, 1998; Schieffelin & Sherman, 1995;

Toor et al., 2015; this study), and heavier individuals spend more time resting (Reeve, 1992; Reeve & Sherman, 1991), one mechanism for locating the new nest location may be to follow the odor of more dominant individuals.

If the nest-locating hypothesis is correct, then I predict that the more dominant an individual is, the more time they will spend in the nest chamber, the more time they will spend close to the nest area, and the less time they will spend in an area far from the nest. I also predict that individuals that are more strongly motivated to return to the nest should show a stronger preference for the odor of dominant colony members. Preliminary analysis of three forthcoming studies designed to test these predictions reveal mixed support for the hypothesis. First, in a 24-hour period, the queen (the most dominant member) of a colony of 12 individuals spent more time in the nest chamber than any other colony member, but there was no correlation between dominance rank and time in the nest chamber when excluding the queen. If the final results reveal a similar pattern, then this prediction of the nest-locating hypothesis would not be met. Second, in a different 24-hour period using the same colony of 12 individuals, there was a correlation between dominance rank and number of visits to an area that was over 5 m away from the nest chamber such that more dominant individuals made fewer visits to the distal area. If the final results of this experiment remain consistent, then this prediction of the nest-locating hypothesis would be met. Third, the current study was repeated for two stimulus pairs (colony 9300, stimulus pair ♀2–♂E; colony 1200B, stimulus pair ♂A–♀7), but this time the subjects were forced to make a choice by isolating them from their stimulus colony and placing them in the T-choice

apparatus. For both stimulus pairs, the preference for the odor from the dominant stimulus donor was not preferred any more strongly, which was predicted if isolation increased motivation to return to the safety of the group. This could either indicate the presence of a ceiling effect or perhaps that the nest-locating hypothesis is not a viable explanation for the preference to approach the odors of dominants.

The current study found that the preference for the odor of the more dominant donor of two fellow colony members was strongest when the dominant donor was a breeder, even when breeders were excluded as subjects. If odor preference does indicate preference to approach, this finding is particularly interesting given that the subjects are potentially approaching individuals that are likely to shove them (Clarke & Faulkes, 2001; Reeve & Sherman, 1991). There are several reasons why non-breeders may nonetheless prefer to approach breeders.

Non-breeders of both sexes may prefer to go towards the odor of the queen particularly because she may decide which chamber the colony is going to use as a nest site ("nest-with-queen hypothesis"). It is unknown how naked mole-rat colonies decide which chamber to nest in, but the queen's presence may be the critical driving force. Since the queen spends more time in the nest than any other colony member (unpublished data), following her odor may help to locate the nest if it moves. Alternative mechanisms may exist for naked mole-rats to locate the nest including following a concentration gradient of amount of any conspecific odors. Further study is needed to test between the hypotheses presented here and other alternatives.

Non-breeders of both sexes may also particularly prefer to go towards the odor

of the queen because they want to be there to help care for the young. Consistent with this possibility, it has been reported that colony members aggregate in the nest more strongly closer to the birth of a litter (Jarvis, 1991; Lacey & Sherman, 1991). Even though pup caring behavior was found to correlate, most often negatively, with body weight (and thus likely dominance; Lacey & Sherman, 1991), the presence of larger more dominant individuals in the nest with pups may contribute to pup rearing in the form of thermoregulation.

Non-breeders of both sexes in colony 1200B showed a very strong preference for the breeder male (♂J), despite the fact that this male was responsible for the vast majority of shoving and shoved the highest number of fellow colony members during casual observations (data not shown). Moreover, it is particularly interesting that the most subordinate member of the colony (♀6) which received the highest number of shoves from the breeder male during casual observations, showed a very strong preference for the breeder male, entering the tube with his odor in 8/8 (100%) trials for stimulus pair ♂J–♀1 and in 7/8 (88%) trials for stimulus pair ♂J–♂X. Whatever the motivation for approaching this breeder male, it is clearly stronger than any possible aversion that may exist to being shoved.

One possible explanation for ♀6's preference for the breeder male is that she may not have been entirely reproductively suppressed and was seeking a mate. Several years after the current study concluded, the queen had died and ♀6, despite being the most subordinate individual within the colony, was one of the females contending to be the new queen. It may be that during the current study, the physiology of ♀6 was

more similar to a non-suppressed individual and that this affected her odor profile which caused her to be frequently attacked by the breeder male in an attempt to keep her from reproducing. Similarly, Margulis and colleagues (1995) observed that a queen directed most of her shoves towards a non-breeder female that was found to exhibit hormonal signs of ovarian activity and was the first to act like a queen after the queen was removed from the colony. Also consistent with this possibility is the fact that ♀6 was shoved by the highest number of other individuals (11/18 colony members, 61%), despite shoving by non-breeders being rare. This may indicate that the breeder male was not the only member of the colony that could smell that she was not as suppressed as she should be.

So, despite the fact that ♀6 was shoved the most by the breeder male, she may have been drawn to him for reproduction. Interestingly, the queen at the time of the current study was never seen shoving ♀6, which may have contributed to her lack of complete suppression. The interest in shoving ♀6 may also have contributed to the lack of preference found for the odor of the more dominant ♂X in stimulus pair ♂X–♀6. It may be that the overall interest in approaching ♀6's odor canceled out the interest in approaching the more dominant ♂X's odor. This seemingly increased preference for ♀6's odor despite her low dominance rank was not seen in stimulus pairs ♂J–♀6 or ♀10–♀6, possibly because ♀6 was paired against the queen or breeder male in each of those pairs and the preference for breeders was strong enough to overcome any possible increased interested in ♀6. Putting aside the possible explanations as to why ♀6 still preferred the breeder male's odor despite often being

shoved by him, it is still the case that other colony members strongly preferred the odor of dominants that were more likely to shove them than subordinates. This suggests that the benefit for approaching the odor of dominants outweighs the potential costs of aggressive interactions.

Another possible explanation as to why breeders and non-breeders of both sexes prefer the odor of the dominant donor is that they are responding to some sort of alarm signal ("alarm-signal hypothesis"). The stress response to being isolated in the stimulus tubes may have been greater for more dominant colony members than less dominant colony members. Consistent with this possibility is the fact that breeders very readily urinated and defecated when removed from the colony while non-breeders did not. If the odor of urine or feces outside of the latrine signals danger, or if breeders released chemicals that signal danger from some unknown odor source, this may explain why the odor of dominants was most strongly preferred when the dominant donor was a breeder. However, since larger (and thus likely more dominant) colony members are more likely to respond to danger in the form of a predator or conspecific intruder (Lacey & Sherman, 1991), one might have predicted that the most dominant and not most subordinate subjects would prefer the odor of dominant donors in order to respond to danger, but preference for the odor of dominants was found for both the subjects in the lowest and highest thirds of the dominance hierarchies. The possibility that naked mole-rats communicate danger via odor is an intriguing possibility and its existence would be yet another similarity this species shares with eusocial invertebrates (Hölldobler & Wilson, 1990).

What odor sources might communicate dominance?

While the results of this study demonstrate that naked mole-rats can discriminate between the odors of fellow colony members based on dominance, the source of the odors being used for discrimination and their chemical nature are still unknown. The current study used "whole-body" odor by isolating stimulus odor donors in the plastic tubes used as arms of a T-choice apparatus. The components of "whole-body" odor include saliva, urine, feces, exudate from various glands, including those of the ano-genital region or bottom of the paws, and flakes of dry skin. Although subjects had direct access to the stimulus odor tubes and any non-volatile chemicals they contained, it is likely that volatile odors and the main olfactory system are what were used when deciding which tube to enter for two main reasons. First, although not systematically recorded, it seemed that in most trials the subject only sniffed the air and did not bring their nose or tongue in contact with the floor or walls of the stimulus tubes. Second, naked mole-rats are unique among rodents in that their vomeronasal neuroepithelium does not grow larger after birth, resulting in a diminished vomeronasal organ compared with other rodents of similar size (Smith, Bhatnagar, Dennis, Morrison, & Park, 2007). However, when naked mole-rats were treated with zinc sulphate to block their main olfactory system, they were still able to discriminate between a familiar and novel stimulus conspecific and show relatively consistent tunnel passing behavior with their fellow colony members, although they may have been able to do so by relying on cues of other modalities like audition (Toor et al.,

2015).

Discrimination of salivary odors based on the dominance of odor donors has been demonstrated in the golden hamster (*Mesocricetus auratus*; Friedle & Fischer, 1984). When naked mole-rats encounter each other face-to-face in a tunnel, saliva seems like a potential odor source that individuals can use to determine dominance and then appropriately climb over a subordinate or allow themselves to be climbed over by a dominant. When the odor donors of this study were isolated in the stimulus tubes, they spent a large portion of their time chewing at the end caps to escape and return to the colony. Although far less common, they also chewed along the inside of the tube wall. Perhaps enough saliva was spread on the tube and end caps to contribute to a subject's ability to discriminate between stimulus tubes, but I find this unlikely for two main reasons. First, the lips of naked mole-rats close behind their incisors, which they use to dig (Hill, Porter, Bloom, Seago, & Southwick, 1957), so they do not have to open their mouth and thus do not likely spread copious amounts of saliva on the surfaces they dig in the way, say, a dog would digging with an open mouth. Secondly, the odors from the rest of a naked mole-rat's body seem more prominent to my human nose, although this may be irrelevant to a naked mole-rat.

The odor of urine can be used to discriminate based on the dominance of odor donors in bank voles (Hoffmeyer, 1982; Kruczek, 1997). However, it is unlikely that the odor of urine played a significant role in the results obtained in this study on naked mole-rats because it was rare that non-breeder odor donors urinated when contained in the stimulus tubes and, if they had, the stimulus tube was replaced. Similarly, fecal

pellets were usually dry and easily removed by shaking out the tube or, if feces were wet, the tube and odor donor was spot wiped clean with water or a different tube was used. Nevertheless, it is possible that trace amounts of urine or feces from the stimulus odor donor was present on their bodies and contributed to the odors of the stimulus arm.

It has been reported that naked mole-rats wallow in their communal latrine and suggested that the function of this behavior is for an individual to acquire their colony's unique olfactory signature and use it to identify foreign intruders (Lacey et al., 1991). If this is the case, it would seem that an individual's body should be covered with a homogenized layer of urine and feces and that the odors of urine or feces coming from an individual should not be individually distinctive or informative as to the individual's dominance rank, and thus not likely used as the basis for discrimination found in the current study. It would further suggest that some other odor source is being used as the basis for discrimination based on dominance and that that odor source can be detected in the presence of a communal odor signature.

However, naked mole-rats do not seem to wallow in the latrine very much, if at all. Several observations suggest that the initial report that naked mole-rats wallow in the latrine may have been made in error. First, I have not observed this behavior in the roughly six years of near-daily care of several naked mole-rat colonies. Second, 24 h of continuous video recordings of the latrine of a single colony did not contain a single clear instance of wallowing (F. R. Castelli, unpublished data). In fact, during many of the visits to the latrine in which subjects urinated, they only ever extended their

hindquarters into the latrine entrance, urinated, and left the area. Third, the function of having a latrine and only urinating and defecating in that part of the tunnel system seems to be one of hygiene and thus wallowing in bodily waste seems counterproductive. In fact, I have seen an entire colony become ill in a matter of hours after an individual who was seemingly eager to dig, removed all the soiled woodchips from the latrine and spread it throughout the tunnel system. Upon discovery, all individuals were immediately bathed and they recovered over a few days. Unfortunately, this happened again to the same colony but by the time it was discovered hours later the entire colony had become very ill and only two of the 14 individuals eventually recovered. Lastly, the wallowing behavior described by Lacey and colleagues (1991) may have been misinterpreted and thus unfortunately labeled. Naked mole-rats perform rolling behaviors in which their dorsal surface comes into contact with the tunnel floor (see *Chapter 4*) and these behaviors may have been mistaken for wallowing when they occurred in or near the latrine. Some rolling behaviors result in the individual having a reversed orientation and may function in changing the direction of movement in a narrow tunnel (e.g., Lacey et al., 1991; see *Chapter 4*). These and other rolling behaviors may also function as a form of scent-marking (see *Chapter 4*). Lacey and colleagues (1991, p. 218) mention that wallowing "typically occurs in the toilet area…," which presumably refers to "…the toilet box or a nearby tunnel…" When naked mole-rats use a latrine in the laboratory, the soiled areas extend overtime out of the latrine box and into the tube leading to the latrine box, apparently due to a buildup of waste from individuals relieving themselves only

at the entrance of the latrine. Therefore, Lacey and colleagues (1991) likely interpreted such rolling behaviors as wallowing in feces and urine when they occurred in the tubes leading to the latrine. Rolling behaviors occur quite frequently, but very rarely have they been observed in a latrine box. If it is the case that naked mole-rats do not actually wallow in the latrine, then perhaps trace amounts of an individual's own urine and feces is present on their body and can be used for discrimination based on dominance by fellow colony members.

Unlike other rodents, naked mole-rats do not have any obvious scent-marking glands on the surface of their body (Tucker, 1981) and no moisture or smeared excreta was detected in the stimulus tubes of the current study. Nevertheless, it is unknown if exudate from glands are odor sources that convey dominance in naked mole-rats and were used by subjects in this study. Sweat can be eliminated from consideration because the skin of naked mole-rats lack sweat glands (Tucker, 1981). Since naked mole-rats are hairless, they also lack the sebaceous glands associated with hair follicles (Daly & Buffenstein, 1998). Naked mole-rats preferentially follow the odor trails of individual foragers that returned from desirable food types and plantar glands have been suggested as a potential source of such trails (Judd & Sherman, 1996), but this has not been confirmed. After urinating in the latrine, sometimes naked mole-rats will drag their ano-genital area on the tube floor as they leave the latrine area, potentially marking with urine or exudate from an ano-genital gland (Lacey et al., 1991), though this too has not been confirmed.

Another potential odor source that may convey dominance is dander, or dry

skin flakes. I have observed that naked mole-rats produce a large amount of dry dead skin on their body surface, despite conditions of very high humidity (3 weeks of >85% relative humidity; F. R. Castelli, unpublished data). When colony tunnel systems are disassembled for routine cleaning, particles can often be shook out of tubes and an accumulation of dead skin (confirmed by microscopic inspection) can be found at the tunnel joints. I hypothesize that this dander may function in olfactory communication and that the rolling behaviors mentioned above are a form of scent-marking to distribute that dander onto the substrate (the topic of *Chapter 4*). This hypothesis is supported by several observations. Many times rolling behavior does not seem to occur simply to allow an animal to change the direction of movement in the tube because: an even number of them may occur in short succession over a short distance resulting in no final change in direction; several may occur in short succession followed by the animal reversing orientation with an upright turn; many will result in a maintained body orientation. Rolling behaviors are also very common. Moreover, anecdotal evidence discussed in *Chapter 4* suggests that the frequency of rolling behaviors may be influenced by dominance rank, a finding that may be corroborated by a forthcoming study. It would seem that if dander cannot be used directly as a cue for dominance rank of the odor donor, it may still communicate dominance rank via the pattern of its deposition in the colony's tunnel system.

How might odor sources convey dominance?

Regardless of the identity of the odor source or sources that naked mole-rats

use to discriminate based on dominance, at least three hypotheses may explain how these odor sources convey dominance. First, the quantity of specific chemical cues within any given sample of these odor sources may correlate with dominance rank ("chemical-correlation hypothesis"). Second, subjects may remember individual odor signatures comprised of multiple chemical cues in different proportions of each colony member and associate these odor signatures with direct, perhaps agonistic, interactions they had with the odor donor which indicate dominance ("individual-interaction hypothesis"). Third, subjects may remember individual odor signatures but associate these signatures with indirect measures of dominance like the frequency or pattern of scent-marking patterns of the odor donor that they regularly encounter in the colony tunnel system ("scent-marking hypothesis").

According to the chemical-correlation hypothesis, the quantity of one or more chemical cues found within an odor source may correlate with dominance rank and be used by individuals to discriminate between odors based on dominance of the odor donor. For example, Gassett and colleagues (1996) identified eleven volatile compounds in the interdigital secretions (used for scent-marking) of white-tailed deer (*Odocoileus virginianus*) that occurred in higher concentrations in dominant than subordinate individuals. In both male and female naked mole-rats, urinary testosterone titers correlate with dominance rank (Clarke & Faulkes, 1997, 1998). The different amounts of testosterone being excreted indicate variation in endocrine physiology based on dominance which may correlate with the quantity of excreted metabolites that directly or indirectly contribute to volatile chemicals that can be used for the basis

of discrimination (e.g., Schwende, Wiesler, Jorgenson, Carmack, & Novotny, 1986). Once the odor source that conveys dominance is identified, it can be chemically analyzed (e.g., Schwende et al., 1986) and presented to subjects in order to figure out the chemical components that are being attended to. Once these chemicals are identified, they can be presented to subjects at different concentrations to see if discrimination and a preference still occurs for what approximates dominant odor.

The chemical correlation-hypothesis posits a possible mechanism for identifying dominance in odors without the need to for a mechanism to identify individual odor donors. Therefore, another way to test this hypothesis is to see if naked mole-rats can discriminate between two unfamiliar individuals from a foreign colony. However, such an experiment may be challenging given that naked mole-rats are very xenophobic (Lacey & Sherman, 1991; O'Riain & Jarvis, 1997) and may react to all foreign naked mole-rat odors with equal repulsion.

In the current study, there was not a significant correlation between the dominance rank difference of a stimulus odor donor pair and the probability of turning towards the odor of the more dominant donor after stimulus pairs including breeders were excluded from analysis. If subjects are drawn to higher concentrations of a chemical that conveys dominance, I would have predicted a stronger preference for the odor of the dominant when the dominance rank difference between stimulus donors was greater. However, dominance rank is on an ordinal scale and an amount of a chemical is a continuous value, so the difference in dominance rank of "1" does not likely correspond to the same difference in the amount of chemical for every

consecutive pair of individuals in the dominance rank hierarchy. Breeders tend to be behaviorally much more dominant than even the most dominant non-breeders as indicated by their shoving rates (Clarke & Faulkes, 2001; Reeve & Sherman, 1991). Similarly, it may be that the chemical amount value is much higher for breeders than the next most dominant non-breeders such that the difference in chemical amount between a dominant breeder stimulus donor and a subordinate non-breeder stimulus donor is much greater than the difference between any two other non-breeder stimulus donors. If the chemical amount for each stimulus donor can be measured before testing, stimulus pairs could be selected such that the difference in amount between the two odor donors allows for a more appropriate test of whether the preference for the odor of the dominant is stronger when the dominance difference with the subordinate donor is greater.

Both the individual-interaction hypothesis and the scent-marking hypothesis posit that naked mole-rats remember unique odor signatures of their fellow colony members and pair those signatures with either memory of direct interactions with the odor donor, or instead, with indirect measures of dominance like scent-marking. Unique individual volatile odor signatures have been identified in other species. For example, the common marmoset (*Callithrix jacchus*) is a cooperative breeder that forms dominance hierarchies and individual females have unique ratios of highly volatile chemicals in their genital secretions that they use for scent-marking (Smith, Tomlinson, Mlotkiewicz, & Abbott, 2001). Similarly, the volatile chemical profiles of naked mole-rat odor sources may be unique to each individual and may also be used as

a basis for discrimination. In a very preliminary exploratory analysis of some volatile chemicals in the feces of three naked mole-rats, the three volatile profiles were individually distinctive (F. R. Castelli and R. A. Raguso, unpublished data). It remains to be seen how variable such profiles are across more individuals and if naked mole-rats attend to these odors. In addition to the existence of unique odor signatures, the individual-interaction hypothesis and the scent-marking hypothesis require an individual to remember these odor signatures as well as the measures of dominance they are to be associated with. This seems like a cognitively demanding task for a naked mole-rat since their colonies in the wild have about 75 individuals on average with the largest colony size recorded at 295 (Brett, 1991b). Nevertheless, perhaps the subjects of the current study were able to use such a mechanism given that test colonies were relative small (colony 9300, $N = 15$; colony 1200B, $N = 19$; colony 1200D, $N = 6$).

The individual-interaction hypothesis posits that naked mole-rats remember unique odor signatures and pair them with memory of direct behavioral interactions they have had with the associated odor donor that would convey dominance. For this hypothesis to be correct, one interesting finding from the current study would need to be explained. Subjects were able to discriminate between the dominant and subordinate odors even when their own dominance rank was not between the ranks of the two odor donors. It is unknown if a naked mole-rat is capable of gathering information on the relative dominance relationship between two fellow colony members from an agonistic interaction that takes place nearby. More likely, an

individual might be able to recognize the dominance rank difference between two fellow colony members based on their own interactions with each of these other individuals. In order for this to work, there would need to be a correlation between a behavioral measure and absolute dominance rank. Agonistic interactions are obvious candidate behaviors for conveying dominance. Individuals may evaluate the strength or fighting ability of fellow colony members on an absolute scale which would enable them to discriminate between two individuals more dominant or two individuals more subordinate than themselves. Very aggressive behaviors seem unlikely for this purpose since they can be infrequent for very long periods of time (Jarvis, 1991). Passing behavior in tunnels would be inappropriate because the frequency of such agonistic interactions would not likely correlate with dominance rank. One promising candidate behavior is incisor fencing (Lacey et al., 1991), during which two individuals lock incisors and pull back and forth or twist their head side to side. Although very energetic instances of this behavior in which both participants are standing up (Lacey et al., 1991) is relatively uncommon (personal observations), I have observed a somewhat relaxed version of this behavior that may be more common in which one or both participants lock incisors and twist their heads, many times while one or both participants are reclining in the nest chamber. Either type of incisor fencing will sometimes lead to one individual falling over onto their side. I speculate that perhaps this behavior is akin to human arm wrestling in that it is a contest of strength or fighting ability and thus a measure of absolute dominance. Individuals may recall how strongly others countered their head twists or how often they were felled. Colonies of

naked mole-rats should be observed to see how common relaxed incisor fencing actually is, if participation is widespread among colony members, and if dominance rank correlates with "winning" such incisor fencing bouts.

The scent-marking hypothesis posits that naked mole-rats remember unique odor signatures and pair them with memory of indirect measures of dominance like scent-marking. *Chapter 4* provides evidence in support for the hypothesis that rolling behaviors in naked mole-rats are a form of scent-marking and, as mentioned above, I suspect these behaviors function to deposit dry skin dander for the purpose of communicating dominance. Naked mole-rat dander may have a volatile chemical signature that is unique to each individual and scent-marking by depositing dander may communicate dominance by the frequency of scent-marks or the pattern of their placement (Gosling & Roberts, 2001). Rolling behavior is very common in laboratory colonies and anecdotal evidence discussed in *Chapter 4* suggests that the frequency of rolling behaviors may be influenced by dominance rank, a finding that may be corroborated by a forthcoming study. Perhaps the extent to which an individual's dander is spread throughout the colony is an honest signal of their strength since engaging in rolling behaviors may be energetically demanding (Gosling & Roberts, 2001). Perhaps this also partly explains why some breeder males fail to maintain a large body weight over time (Jarvis et al., 1991); they burn many calories rolling. The subjects of the current study may therefore have associated an odor donor's individual odor signature with memory of how widely spread that individual's odor was throughout the colony tunnel system, an indirect measure of dominance.

Conclusions

The results of this study support the hypothesis that naked mole-rats are able to discriminate between the odors of fellow colony members based on the dominance status of the odor donors and it was revealed that the odor of the more dominant donor is most often preferred. Furthermore, this pattern of results remained consistent when subjects were analyzed separately according to sex, breeding status, their absolute dominance rank, and their dominance rank relative to stimulus odor donors, and when stimulus odor donor pairs varied according to dominance status, sex, breeding status, and body weight. These results generate questions for further testing using naked mole-rats including the following: the identity of the odor sources that convey dominance, how these odors convey dominance, how the ability to discriminate between odors based on dominance helps to maximize fitness, and why the odors of dominant donors are preferred. By better understanding how individuals in naked mole-rat colonies interact and communicate, we can gain insight as to how their unusual eusocial cooperative breeding system has evolved.

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CHAPTER 4

ROLLING BEHAVIOR AS A FORM OF SCENT-MARKING IN NAKED MOLE-RATS (*HETEROCEPHALUS GLABER*)

ABSTRACT

Naked mole-rats are eusocial fossorial rodents that perform rolling behaviors in which an individual's dorsal surface makes contact with the floor of a tunnel. In this study, four different rolling behaviors were observed: the flip-twist described by Lacey and colleagues (1991) and the newly described twist-situp, the flip-situp, and the twist-twist. The latter two maintain body orientation, while the former two reverse it, leading to the possibility that some rolling behaviors function in changing the direction of movement. However, several observations suggest that rolling behaviors may function in olfactory communication as a form of scent-marking. This study tests the *rolling as scent-marking* hypothesis. In three colonies of naked mole-rats, a plastic tube of each colony was replaced on alternating days with a clean tube, to stimulate scent-marking, or a "dirty" tube that had been part of the colony tunnel system for 24 h prior, and rolling behaviors and upright turns were scored for 2.75 h each day for 20 days. As predicted, rolling behaviors were more frequent in the clean than the dirty stimulus tube condition. This difference was statistically significant in analyses of: all four rolling behaviors pooled together; only rolling behaviors that reverse orientation; only rolling behaviors that maintain orientation; only rolling behaviors that begin with a flip versus a twist; the flip-twist alone, and the flip-situp alone. Upright turns seemed

to be the preferred method to reverse orientation as they occurred much more frequently than rolling behaviors that reverse orientation. Also as predicted, the frequency of upright turns did not differ between the clean and dirty stimulus tube conditions. A very pregnant queen was physically unable to turn while upright, but could flip-twist, suggesting that at least the flip-twist behavior may function in changing the direction of movement in certain contexts. Lastly, as predicted, the frequency of rolling behaviors or upright turns did not differ based on sex. Dry skin dander as a possible odor source deposited by rolling behaviors and several avenues for future research are discussed.

INTRODUCTION

Naked mole-rats (*Heterocephalus glaber*) are eusocial rodents that live in mixed-sex colonies of about 75 individuals, on average, usually with only a single breeder female (the "queen") and 1-3 breeder males at any given time (Braude, 2000; Brett, 1991b; Jarvis, 1981; Lacey & Sherman, 1991; Sherman, Jarvis, & Braude, 1992). They are a fossorial species with a vast network of underground tunnels and chambers formed as they cooperatively dig for food in the form of tubers and other underground plant matter (Brett, 1991a). Several morphological adaptations aid in their subterranean survival, including loose skin, short limbs, and a cylindrical body which likely help in navigating narrow tunnels (Brett, 1991a). Specific behaviors are also likely to have evolved to negotiate narrow tunnels including those that aid in reversing orientation to change the direction of locomotion. Lacey and colleagues

(1991) were the first to describe a rolling behavior of naked mole-rats that they believe serves this function: "*Turning* animals can reverse the direction of locomotion by completing a forward somersault [Henceforth referred to as a "flip"] followed by a 180° twist, such that the ventral portion of the body [ultimately] faces the substrate" (p. 233). While it is obvious that this behavioral sequence results in a reverse orientation, and this behavior is commonly observed in conjunction with the enacting individual changing their direction of locomotion, several observations suggest that this rolling behavior may not always be used for this purpose.

First, in a laboratory tunnel system composed of plastic tubes with sufficiently wide inner diameters, naked mole-rats can efficiently and often smoothly reverse orientation by turning around while remaining upright, which seems to take less time to execute and is more often successfully executed than the "flip-twist" rolling behavior described by Lacey and colleagues (1991). Moreover, this appears to be the more common method for reversing orientation in laboratory tubes. Second, the flip-twist rolling behavior is observed in areas other than tubes, specifically, and commonly, in corner joints that connect two tubes and even sometimes in voluminous nest boxes. Both joints and boxes are more spacious than tubes and allow individuals to reverse orientation in other ways, which are frequently observed, including backing into the corner and then moving forward in another direction or simply turning and moving forward in any direction. Third, since naked mole-rats seem to memorize their colony's tunnel configuration and readily walk or run backwards apparently as easily and as swiftly as they can move forwards, it is puzzling to see them reverse orientation

by performing a flip-twist and then only advancing a short distance before stopping. If an individual only wanted to go back a short distance, why not simply walk backwards? Fourth, similarly, if an individual has not advanced very far into a tube and is thus not far from a joint or box, why not walk backwards into the joint or box where they can turn around more easily? Fifth, sometimes multiple flip-twist behaviors occur in rapid succession over a short distance and ultimately result in the individual maintaining their original orientation. It seems unlikely that this reflects a bout of fickleness. Furthermore, these bouts of flip-twist behaviors may shortly proceed or be succeeded by upright turns which seemingly could have been executed in the first place if the goal was to reverse orientation. Finally, there are other rolling behaviors that are similar to the flip-twist but do not actually result in a reverse orientation. Perhaps all of these rolling behaviors serve the same function, an alternative to the function of changing the direction of movement in the narrow confines of a tunnel.

I propose that rolling behaviors of naked mole-rats may function in olfactory communication as a form of scent-marking.

As a nearly blind rodent (Hetling et al., 2005), naked mole-rats rely on other modalities of communication including olfaction. They are highly attuned to social odors. For example, individuals prefer the odor of soiled bedding and used nesting material of their own colony versus another colony and will even attack a fellow colony member that had been scented with odors from a foreign colony (O'Riain & Jarvis, 1997). Additionally, naked mole-rats preferentially follow the odor trail of

another colony member that returned to the nest with food to one that did not (Judd & Sherman, 1996). Furthermore, naked mole-rats are able to discriminate between the odors of fellow colony members based on the dominance of odor donors (see *Chapter 2*). Within a naked mole-rat colony, scent-marking may serve several functions including the communication of an individual's continued presence in the colony or the communication of an individual's dominance via chemical cues in found in the odors or the pattern of odor deposition throughout the colony (see *Chapter 2*).

Naked mole-rat rolling behaviors are somewhat similar to scent-marking behaviors of other rodent species. For example, Steiner (1974) describes "*Rubbing with Body-twisting ("Twist-marking"), or Rolling on the Back*" (p. 890), a behavior common in ground squirrel species (e.g., *Urocitellus* spp. and *Callospermophilus* spp.; Kivett, Murie, & Steiner, 1976; Steiner, 1974). Ground squirrels have a collection of apocrine glands on their dorsal surface that serve communicative functions (Mateo, 2002, 2003, 2006), and rolling serves to distribute gland exudate onto the substrate (Kivett et al., 1976). Similarly, kangaroo rats (*Dipodomys* spp.) roll their bodies in sand ("sandbathing"; Eisenberg, 1963), which deposits exudate from specialized sebaceous holocrine skin glands on their backs (Quay, 1953, 1954) that also function in olfactory communication (Laine & Griswold, 1976; Randall, 1981, 1987). Naked mole-rats, in contrast, do not appear to have scent glands on their dorsa, as no gland is macroscopically visible nor does any exudate appear on their skin which remains quite dry. Moreover, naked mole-rats lack sweat glands (Tucker, 1981) and, being hairless, they lack the sebaceous glands associated with hair follicles (Daly & Buffenstein,

1998). However, naked mole-rats shed a fair amount of dry skin dander that may be used in olfactory communication and could be deposited on tunnel floors by rolling behaviors (see *Chapter 2*).

The goal of the current study was to test the *rolling as scent-marking* hypothesis that rolling behaviors of naked mole-rats function in olfactory communication as a form of scent-marking. Using three colonies of naked mole-rats, a plastic tube of each colony was replaced on alternating days with a clean tube or a "dirty" tube that had been part of the colony tunnel system for 24 h prior, and rolling behaviors and upright turns were scored for 2.75 h each day for 20 days. It was predicted that rolling behaviors, regardless of subject sex, would occur more often under the clean than the dirty stimulus tube condition because subjects should be more motivated to deposit their scents in areas deficient in naked mole-rat odor. It was also predicted that upright turns, regardless of subject sex, would not differ between the two stimulus tube conditions because the motivation of subjects to reverse orientation should not be influenced by the level of naked mole-rat odor present in the stimulus tube.

Four different rolling behaviors were observed and described: the flip-twist of Lacey and colleagues (1991) and the herein newly described twist-situp, flip-situp, and twist-twist rolling behaviors, of which the former two result in a reverse orientation and the latter two a maintained orientation. As predicted, for most analyses, rolling behaviors were significantly more frequent in the clean than the dirty stimulus tube condition. Upright turns seemed to be the preferred method to reverse orientation as

they occurred much more frequently than rolling behaviors that reverse orientation. Also as predicted, the frequency of upright turns did not differ between the clean and dirty stimulus tube conditions. Lastly, as predicted, subject sex did not have a significant effect on rolling behaviors or upright turns. Overall, the current study lends support to the *rolling as scent-marking* hypothesis.

METHODS

Animal husbandry

Three colonies of naked mole-rats (*Heterocephalus glaber*) totaling 29 individuals were used in this study. Animals were housed in the laboratory of Dr. Paul Sherman (Mudd Hall, Department of Neurobiology and Behavior, Cornell University, Ithaca, NY). Each colony was housed in a separate tunnel system of clear polycarbonate tubes (5 cm diameter), acrylic boxes (20 x 20 x 12 cm, L x W x H), and polyvinyl chloride (PVC) plumbing joints. The tubes and boxes were transparent and the joints had clear plastic windows to allow for observation. Because naked mole-rats are a fossorial species, the colonies were housed without white light in two separate climate-controlled rooms (~28°C, ~40% RH). To reduce disturbances, tunnel systems rested upon foam-lined plywood suspended on inflated rubber inner tubes. Red desk lamps with incandescent bulbs were positioned a few inches above parts of the colony including the nest chamber to provide additional heat and to allow for observation. Naked mole-rats obtain water from their food. A mixture of fruits and vegetables were provided fresh daily such that some food remained unfinished the following day. The

most commonly provided food items were sweet potatoes, carrots, green beans, lettuce, banana, pear, and apple. Occasional food items included jicama and grapes. Naked mole-rats usually used one chamber of their tunnel system as a latrine which was cleaned and furnished with aspen wood shavings daily. Some clean shavings were removed by the mole-rats to line their nest chamber.

The identity of individuals was tracked over the course of the study period by a combination of body weight, toe clippings, and distinguishing body characteristics such as tattoo scars, tail length, and overall body shape. To allow for easy observation, a black marker was used to write a letter or number on each individual's dorsal surface which was re-applied as needed, usually to all individuals of a colony on the same day.

Study animals

Twenty-nine naked mole-rats (N = 21 males, 8 females) from three different colonies were used in this study: colony "1200B-2" (N = 10 total; 8 males; 2 females); colony "1200C" (N = 8 total; 7 males; 1 female); and colony "1200E" (N = 11 total; 6 males; 5 females). All three colonies were formed by removing individuals from colony "1200" (not used in this study). Due to a partial loss of breeding records, details such as breeding history, age, and relatedness are incomplete for these two colonies. Breeder females ("queens") were the only females to become pregnant and breeder males (1 in each of these colonies) were determined by observation of copulation and a high frequency of mutual ano-genital nuzzling, characteristic of breeders (Ciszek, 2000; Faulkes, Abbott, Liddell, George, & Jarvis, 1991; Jarvis,

1991; Lacey et al., 1991; Lacey & Sherman, 1991). The queen of colony 1200B-2 had most recently given birth to a litter four days before the start of the current study, though no pups survived after two days, prior to data collection. The queen of colony 1200C was pregnant throughout the current study and gave birth to a litter of 18 pups two days after the study ended. In colony 1200E, the queen had given birth to a litter three months prior to the current study and then subsequently about one month after the study ended, though no pups were present in the colony during the study.

Procedures

Colony tunnel systems were cleaned 2-5 d prior to the start of the study. For each colony, a 61 cm segment of plastic tubing, the "stimulus tube," was removed and immediately replaced with either a dry clean tube or the same "dirty" tube on alternating days for 20 consecutive days for colonies 1200B-2 and 1200E. For colony 1200C, the second day of data collection was nine days after the first and then data collection proceed consecutively for the remaining days. A line was drawn with permanent marker lengthwise along the bottom of each tube to preserve orientation after replacement. Tubes were cleaned with soap and hot water using a round scrub brush that fit inside the tubes, then rinsed with 50% ethanol followed by a rinse with deionized tap water. After cleaning, clean tubes were placed in the colony room unattached to any tunnel system for 24 h so that they would equilibrate to colony room temperature prior to use. Tubes were secured at both ends to pieces of PVC plumbing pipe joints using threaded bolts. To control for disturbance of each colony during

testing days, tube removal and replacement was done no longer than 45 min after the colony was provided fresh food and the latrine was cleaned. Aside from the stimulus tube, only the latrine and the tube connecting the latrine to the rest of the colony tunnel system were ever cleaned throughout the study. A tripod-mounted camera was used to video-record the stimulus tube from the moment it was placed for 2.75 h. Video footage was scored by an observer who was blind to stimulus tube condition. Permanent marker was used to mark a ring around each stimulus tube 5 cm from both ends and behaviors that occurred in the middle 51 cm were scored.

Behaviors scored

Behaviors for which an individual's dorsal surface made contact with the floor of the stimulus tube are referred to as "rolling" behaviors or "rolls." Four types of rolling behaviors were identified and scored, and their descriptions can be found in Table 4.1. Each rolling behavior is made up of two component behaviors. The first component behavior is what causes the individual's dorsum to come into contact with the floor. The second component behavior is how the individual gets up off the floor and returns to an upright position. The flip-twist and twist-situp both result in a reverse orientation, while the flip-situp and twist-twist maintain the individual's original orientation. Sometimes individuals would lower their head to the floor and begin to perform a flip but would be interrupted by a passing colony member or a sudden switch in behavior, for example, the apparent scratching of an itch; these instances were scored as "incomplete" rolls because the individual clearly started to roll but did

not complete the behavior. Also scored were “upright turns,” when an individual remained upright on all four limbs while reversing their orientation, usually in a forward direction but occasionally by moving backwards.

Table 4.1. Descriptions of the four rolling behaviors of naked mole-rats (*Heterocephalus glaber*) that occurred in a stimulus tube for 2.75 h after it was placed in the colony tunnel system after it had either been cleaned ("clean") or had been part of the tunnel system for 24 h prior ("dirty"). Observations were made of three colonies (colony 1200B-2: $N = 10$ subjects, colony 1200C: $N = 8$ subjects, and colony 1200E: $N = 11$ subjects). Each rolling behavior is made up of two component behaviors: the first is what causes the individual's dorsum to come into contact with the floor; the second is how the individual returns to an upright position.

Rolling behavior	first component	description	second component	description	resulting orientation
flip-twist	flip-	The head is tucked in and lowered to the floor as the rest of the body moves forward such that the dorsum (always the back of the head/neck, but usually the entire back) lands on the floor.	-twist	From an inverted position, the body remains straight on the anterior-posterior access and the torso rotates (in either direction) by twisting from anterior to posterior to invert the body such that the forelimbs followed by the hindlimbs make contact with the floor and the animal returns to the upright position.	reverse
flip-situp			-situp	The head is tucked in and lifted and abductor muscles are flexed while curving the spine until the forelimbs are supporting the anterior portion of the body after which the body moves forward until standing on all four limbs.	maintained
twist-twist	twist-	The body remains striaght on the anterior-posterior access and torso rotates (in either direction) by twisting from anterior to posterior to invert the body such that the dorsum makes contact with the floor.	-twist	From an inverted position, the body remains straight on the anterior-posterior access and the torso rotates (in either direction) by twisting from anterior to posterior to invert the body such that the forelimbs followed by the hindlimbs make contact with the floor and the animal returns to the upright position.	maintained
twist-situp			-situp	The head is tucked in and lifted and abductor muscles are flexed while curving the spine until the forelimbs are supporting the anterior portion of the body after which the body moves forward until standing on all four limbs.	reverse

Statistical Analysis

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, U.S.A.). Statistical significance was set at $P < 0.05$ and marginally non-significant trends were noted when $0.05 \leq P \leq 0.10$.

Counts of behaviors in the stimulus tube were analyzed using generalized estimating equations (GEE) negative binomial regression models with stimulus tube condition (clean or dirty) as the predictor variable and subject as a cluster variable modeled with an exchangeable correlation structure within subjects to control for repeated measures (PROC GENMOD, SAS). A nested correlation structure of individual within colony was not necessary in analyses of pooled data from multiple colonies, so only subject was specified in the repeated statement option. This is sufficient because the statement only needs to distinguish correlated observations (those from the same subject) from uncorrelated ones because the GEE method is robust ("SAS Usage Note 24200," 2004). Type III tests for significance of fixed effects were calculated using the default score statistics for GEE. Estimate LS-means are reported on the inverse linked scale as frequency of behavior with 95% confidence intervals. To see if males and females behaved differently, additional models were run as above with subject sex, stimulus tube condition, and their interaction as the predictor variables. Since the interaction term was not significant for all those models, they were rerun without it and the results were reported as the effects of subject sex and stimulus tube condition.

Ethical Note

The methods used were approved by Cornell University's Institutional Animal Care and Use Committee. At the end of the study, animals remained in the colony for use in other studies.

RESULTS

Rolling Behaviors

The frequencies and proportional breakdowns of the four types of rolling behaviors that were observed are presented in Table 4.2 and statistical analyses of the effect of stimulus tube condition (clean or dirty) on each rolling behavior and on several combinations of rolling behaviors are presented in Table 4.3. Rolling behavior was observed being performed by almost all subjects; 27 out of 29 subjects successfully executed at least one rolling behavior of any type (Table 4.2). As predicted, when all four rolling behaviors were analyzed together, the frequency of rolling behaviors was significantly greater when the stimulus tube was clean than when it was dirty ($P = 0.007$; Table 4.3). This result was consistent when incomplete rolls were included in the analysis ($P = 0.007$; Table 4.3).

The flip-twist, originally described by Lacey and colleagues (1991) and results in reverse orientation, was the most frequently observed rolling behavior and observed being performed by the highest number of subjects, followed by the flip-situp rolling behavior, which maintains orientation (Table 4.2). Rolling behaviors were also significantly more frequent in clean stimulus tubes than dirty tubes when analyzing

either of these two rolling behaviors separately (flip-twist: $P = 0.006$; flip-situp: $P = 0.038$) or together as rolling behaviors that begin with a flip ($P = 0.007$), whether or not incomplete rolls were included in the analysis ($P = 0.007$; Table 4.3). There was a non-significant trend for incomplete rolls, which always began with the start of a flip, to occur more frequently in the clean than the dirty stimulus tube condition ($P = 0.090$; Table 4.3).

The twist-twist and twist-situp rolling behaviors, which result in a maintained and reverse orientation, respectively, did not significantly differ between stimulus tube conditions whether or not they were analyzed together or separately ($P \geq 0.152$; Table 4.3), likely owing to their infrequent occurrence and having been performed by the fewest number of subjects (Table 4.2). Most (28/43, 65%) of the twist-twist behaviors were performed by only two individuals: the breeder male of colony 1200C (14 in clean stimulus tube condition; 4 in dirty stimulus tube condition) and the only non-breeding female of colony 1200B-2 (10 in clean stimulus tube condition; 0 in dirty stimulus tube condition). The individual with the highest frequency of the twist-situp behavior was the breeder male of 1200C who was responsible for 12/26 (46%) instances, eight in the clean and four in the dirty stimulus tube condition.

Table 4.2. Frequencies and proportional breakdowns of rolling behaviors, the frequency of upright turns, and the frequency of the 29 naked mole-rat (*Heterocephalus glaber*) subjects that performed each of these behaviors in a stimulus tube for 2.75 h after it was placed in the colony tunnel system, whether or not it had been cleaned or had been part of the tunnel system for 24 h prior. See Table 4.1 for full descriptions of each type of behavior. These data are pooled from three colonies (colony 1200B-2: $N = 10$ subjects, colony 1200C: $N = 8$ subjects, and colony 1200E: $N = 11$ subjects).Proportional breakdowns of rolling behaviors are based on the total frequency of rolling behaviors (2204); upright turns were tallied separately.

		Behavior		Subjects	
Index	Rolling behavior	frequency	%	frequency	% of 29
1	flip-twist	1715	77.8%	23	79.3%
2	flip-situp	261	11.8%	16	55.2%
3	twist-twist	43	2.0%	11	37.9%
4	twist-situp	26	1.2%	6	20.7%
5	incomplete	159	7.2%	22	75.9%
1,2,3,4,5	all types, including incomplete	2204	100.0%	28	96.6%
1,2,3,4	all types, excluding incomplete	2045	92.8%	27	93.1%
1,2,5	flip first types, including incomplete	2135	96.9%	25	86.2%
1,2	flip first types, excluding incomplete	1976	89.7%	23	79.3%
3,4	twist first types	69	3.1%	12	41.4%
1,4	types that reverse orientation	1741	79.0%	23	79.3%
2,3	types that maintain orientation	304	13.8%	20	69.0%
	upright turns that reverse orientation	2966	-	28	96.6%

Table 4.3. Mean frequencies with 95% confidence intervals of rolling behaviors in naked mole-rats (*Heterocephalus glaber*) that occurred in a stimulus tube for 2.75 h after it was placed in the colony tunnel system after it had either been cleaned ("clean") or had been part of the tunnel system for 24 h prior ("dirty") and the results of repeated measures negative binomial regression models analyzing each behavioral category. See Table 4.1 for full descriptions of each type of behavior. These data are pooled from three colonies (colony 1200B-2: $N = 10$ subjects, colony 1200C: $N = 8$ subjects, and colony 1200E: $N = 11$ subjects). Bars represent mean frequencies with 95% confidence intervals converted from log LS-means. *P* values indicate significant (*) or marginally nonsignificant (†) differences of LS-means.

Index	Rolling behavior	mean frequency (95% CI)		effect of stimulus tube	
		clean	dirty	χ^2 *(df = 1)*	*P*
1	flip-twist	4.58 (2.74, 7.64)	1.34 (0.88, 2.04)	7.50	*0.006
2	flip-situp	0.61 (0.33, 1.10)	0.29 (0.17, 0.52)	4.29	*0.038
3	twist-twist	0.11 (0.04, 0.30)	0.04 (0.02, 0.10)	2.05	0.152
4	twist-situp	0.05 (0.02, 0.15)	0.04 (0.01, 0.11)	0.67	0.414
5	incomplete	0.42 (0.18, 0.97)	0.13 (0.05, 0.31)	2.88	†0.090
1,2,3,4,5	all types, including incomplete	5.77 (3.49, 9.54)	1.83 (1.23, 2.73)	7.36	*0.007
1,2,3,4	all types, excluding incomplete	5.34 (3.22, 8.88)	1.71 (1.14, 2.56)	7.37	*0.007
1,2	flip first types, excluding incomplete	5.18 (3.10, 8.66)	1.63 (1.08, 2.46)	7.30	*0.007
1,2,5	flip first types, including incomplete	5.60 (3.37, 9.32)	1.76 (1.17, 2.63)	7.29	*0.007
3,4	twist first types	0.16 (0.06, 0.43)	0.08 (0.03, 0.19)	1.95	0.163

When analyzed together, the two rolling behaviors that result in reverse orientation, the flip-twist and twist-situp, were significantly more frequent in the clean than the dirty stimulus tube condition ($P = 0.006$; Figure 4.1). Similarly, the two rolling behaviors that result in a maintained orientation, the flip-situp and twist-twist, were also significantly more frequent in the clean than the dirty stimulus condition ($P = 0.027$; Figure 4.1).

Upright turns

In confirmation of casual observations, compared to rolling behaviors, upright turns appear to be the preferred method to reverse orientation. All subjects were observed performing an upright turn at least once, except for the very pregnant queen of colony 1200C who was physically incapable of doing so (Table 4.2). The frequency of upright turns, which allow individuals to reverse orientation without rolling onto the floor, was about 70% higher than the total frequency of rolling behaviors that result in reverse orientation (flip-twist and twist-situp) and about 35% higher than the total frequency of all four rolling behaviors and incomplete rolls (Table 4.2). As predicted, the frequency of upright turns was not significantly different between the clean and dirty stimulus tube conditions ($P = 0.302$; Figure 4.1).

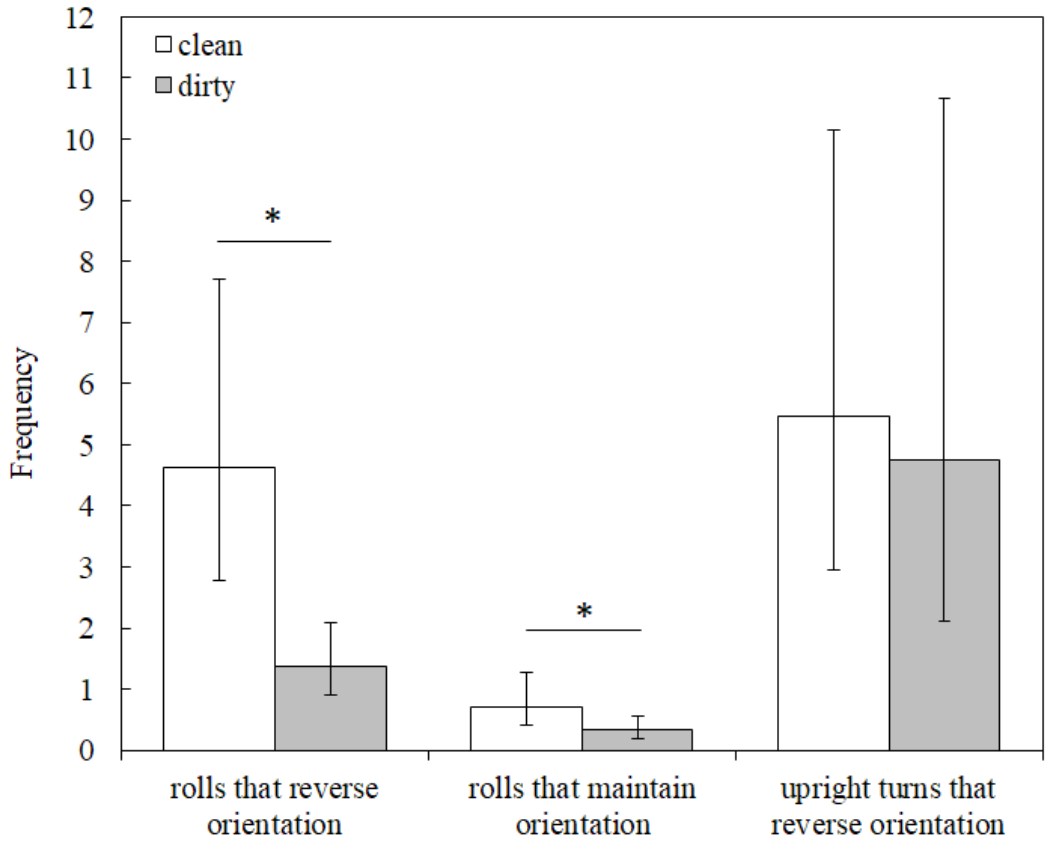

Figure 4.1. Frequencies of rolling behaviors ("rolls") and upright turns in naked mole-rats (*Heterocephalus glaber*) that occurred in a stimulus tube for 2.75 h after it was placed in the colony tunnel system after it had either been cleaned ("clean") or had been part of the tunnel system for 24 h prior ("dirty"). Rolling behaviors are categorized as those that result in the subject being in a reverse orientation (the "flip-twist" and "twist-situp" behaviors; see text for descriptions; Repeated measures negative binomial regression: $\chi_1^2 = 7.53$, $P = 0.006$) and those that result in the subject maintaining their original orientation ("flip-situp" and "twist-twist"; see text for descriptions; Repeated measures negative binomial regression: $\chi_1^2 = 4.90$, $P = 0.027$) upon the completion of the behavior. Upright turns are when subjects reverse their orientation by turning around while remaining upright on all four limbs (Repeated measures negative binomial regression: $\chi_1^2 = 1.07$, $P = 0.302$). These data are pooled from three colonies (colony 1200B-2: $N = 10$ subjects, colony 1200C: $N = 8$ subjects, and colony 1200E: $N = 11$ subjects). Bars represent mean frequencies with 95% confidence intervals converted from log LS-means. Horizontal bars with an asterisk (*) denote significant differences between clean and dirty stimulus tubes.

Effect of subject sex

To see if the sex of a subject had a significant effect on rolling behaviors and upright turns, all analyses were rerun with statistical models including subject sex and stimulus tube condition (clean or dirty) as predictor variables. No analysis showed a significant effect of subject sex, and including subject sex in the model did not change the significance or lack of significance of the effect of stimulus tube condition in any analysis (Table 4.4). There was a non-significant trend for the frequency of flip-situp rolling behavior to be higher in males (0.53 flip-situp rolling behaviors [0.30, 0.92]) than females (0.15 flip-situp rolling behaviors [0.04, 0.59]; $P = 0.068$; Table 4.4). Analysis of the twist-situp rolling behavior was not possible due to zero instances of females performing this behavior, although six males were observed performing this behavior 26 times (clean = 15 instances, dirty = 11 instances).

Table 4.4. The results of repeated measures negative binomial regression models analyzing the effects of stimulus tube condition and subject sex on the frequency of rolling behaviors and upright turns in naked mole-rats (*Heterocephalus glaber*) that occurred in a stimulus tube for 2.75 h after it was placed in the colony tunnel system after it had either been cleaned ("clean") or had been part of the tunnel system for 24 h prior ("dirty"). See Table 4.1 for full descriptions of each type of behavior. Upright turns are when subjects reverse their orientation by turning around while remaining upright on all four limbs. These data are pooled from three colonies (colony 1200B-2: N = 10 total, 8 males, 2 females; colony 1200C: N = 8 total, 7 males, 1 female; and colony 1200E: N = 11 total, 6 males, 5 females; sum of colonies: N = 29 total, 21 males, 8 females). P values indicate significant (*) or marginally nonsignificant (†) differences of LS-means. The analysis for "twist-situp" behavior was not possible due to zero instances of females performing this behavior, although males were observed performing this behavior 26 times (clean = 15 instances, dirty = 11 instances).

		effect of subject sex		effect of stimulus tube	
Index	Rolling behaviors	χ^2 *(df = 1)*	*P*	χ^2 *(df = 1)*	*P*
1	flip-twist	1.06	0.304	7.32	*0.007
2	flip-situp	3.33	†0.068	4.51	*0.034
3	twist-twist	0.02	0.895	2.10	0.147
4	twist-situp	-	-	-	-
5	incomplete	1.07	0.302	3.20	*0.074
1,2,3,4,5	all types, including incomplete	1.59	0.208	7.01	*0.008
1,2,3,4	all types, excluding incomplete	1.47	0.226	7.04	*0.008
1,2,5	flip first types, including incomplete	1.56	0.212	7.06	*0.008
1,2	flip first types, excluding incomplete	1.44	0.231	7.09	*0.008
3,4	twist first types	0.37	0.541	1.82	0.178
1,4	types that reverse orientation	1.15	0.283	7.32	*0.007
2,3	types that maintain orientation	2.36	0.124	4.29	*0.038
	upright turns that reverse orientation	0.82	0.366	1.24	0.265

DISCUSSION

Summary of results

Overall, the results lend support to the *rolling as scent-marking* hypothesis that rolling behaviors of naked mole-rats function in olfactory communication as a form of scent-marking. Four different rolling behaviors were observed and described: two that reverse orientation, the flip-twist of Lacey and colleagues (1991) and the newly described twist-situp; and two that maintain orientation, the flip-situp and the twist-twist, also both described for the first time. As predicted, rolling behaviors were more frequent in the clean than the dirty stimulus tube condition. This difference was statistically significant in analyses of: all four rolling behaviors pooled together; only rolling behaviors that reverse orientation; only rolling behaviors that maintain orientation; and only rolling behaviors that begin with a flip versus a twist. This difference was also statistically significant for two of the four rolling behaviors observed when analyzed separately (the flip-twist and the flip-situp). Incomplete rolls were also observed, but analyses were consistent whether or not they were included with or excluded from the appropriate analyses. The frequency of the twist-twist rolling behavior, which maintains orientation, and the twist-situp rolling behavior, which reverses orientation, were not significantly different between stimulus tube conditions whether analyzed separately or together, likely due to their infrequent occurrences.

Also as predicted, the frequency of upright turns did not differ between the clean and dirty stimulus tube conditions, despite the fact that they were more frequent than the total number of all rolling behaviors, whether or not those rolling behaviors

reverse or maintain orientation. Lastly, as predicted, subject sex did not have an effect on any behaviors measured.

Scent-marking versus changing the direction of movement

The current study lends support to the *rolling as scent-marking* hypothesis that rolling behaviors of naked mole-rats function in olfactory communication as a form of scent-marking. As predicted, most analyses of rolling behaviors showed a statistically significant difference between stimulus tube conditions, with a higher frequency of rolling behaviors having occurred in the clean than the dirty stimulus tube condition. The likely interpretation of these results is that subjects were more motivated to deposit their odor in a clean stimulus tube that lacked naked mole-rat odor. Although all members of the colony could access the stimulus tube at the start of each testing period and subjects often entered it immediately, the overall amount of naked mole-rat odor that accumulated in the stimulus tube by the end of the clean testing period would have been much less than the amount that had accumulated by the start of the dirty testing period, which took place after an additional 22.5 h of exposure to the test colony.

Separate analyses of rolling behaviors that reverse orientation and those that maintain orientation both revealed a significantly higher frequency of rolling in the clean than dirty stimulus tube condition. Certainly those rolling behaviors that maintain an individual's orientation upon completion, the flip-situp and twist-twist rolling behaviors, cannot function in changing the direction of movement in a tunnel.

It is therefore more likely that these two kinds of rolling behaviors are both scent-marking behaviors. The flip-situp behavior was more common than the twist-twist behavior and was the second-most common rolling behavior overall. It also occurred significantly more frequently in the clean than the dirty stimulus tube condition when analyzed alone. In contrast, the twist-twist behavior was considerably rarer, and possibly because of this, the tendency for a higher frequency (2.8x more) in the clean than the dirty stimulus tube condition was not statistically significant. An analysis of the twist-twist behavior at the level of the individual found that most of the twist-twist behaviors were performed by only two individuals, both of whom showed a higher frequency of the behavior in the clean than the dirty stimulus tube condition.

The flip-twist and twist-situp rolling behaviors result in reverse orientation and thus seem to possibly function in changing the direction of movement in a tunnel. Other fossorial species may have evolved similar behaviors to navigate narrow tunnels. For example, when describing the digging behavior of moles of the family Talpidae, phylogenetically distant from naked mole-rats, Gorman and Stone (1990) write, "…the mole turns within the tight confines of the tunnel, either sideways [an upright turn] or by somersaulting [a rolling behavior]…" (p. 19). There is mixed support that rolling behaviors function in changing direction in naked mole-rats. If the flip-twist behavior is for the purpose of changing the direction of movement, then why did it occur significantly more frequently in the clean than the dirty stimulus tube condition? Aside from the interpretation that this behavior is a form of scent-marking, perhaps subjects were simply more likely to perform this behavior incidentally in

clean stimulus tubes due to an increased motivation to investigate and spend more time in them because they lack naked mole-rat odor. The amount of time subjects spent within the stimulus tubes was not scored, but two facts taken together suggest that this is an unlikely explanation: first, the frequency of upright turns was about 1.7 times greater than the flip-twist behavior, possibly indicating a preference for upright turns as the method for changing direction in a tunnel; and second, the difference in frequency of upright turns between the clean and dirty stimulus tube conditions was not statistically significant. If the occurrences of subjects wanting to change direction were higher in the clean tube condition because the subjects incidentally spent more time there, then one would have expected a significantly greater number of upright turns as well as flip-twist behaviors in the clean stimulus tube condition, but this was only true for the latter.

Although the twist-situp rolling behavior results in reverse orientation and occurred at similar frequencies in the clean and dirty stimulus tube conditions, the evidence that this behavior functions in changing the direction of movement is not very strong. This was the rarest of the four rolling behaviors observed, occurring least frequently (1.2% of rolling behaviors) and being performed by the fewest number of individuals, a scanty six out of 29 (21%) subjects. If the function of this behavior is to change the direction of movement, it seems that both the flip-twist and upright turn behaviors are far more strongly preferred for this purpose and that this behavior may be idiosyncratic to a few individuals. Interestingly, the individual that performed the highest number of twist-situp behaviors was responsible for almost half of the

occurrences and performed twice as many in the clean as the dirty stimulus tube condition, lending some support to the *rolling as scent-marking* hypothesis. Further testing is needed to see if the two rolling behaviors that maintain orientation serve subtly different functions in changing the direction of movement or in scent-marking.

It is possible that rolling behaviors that reverse orientation function in both scent-marking and changing the direction of movement in a tunnel, depending on the context and motivation of the individual. In the current study, the clean stimulus tube condition was intended to increase motivation for scent-marking behavior, and the use of stimulus tubes with inner diameters that allowed for unencumbered upright turning behavior may have decreased the need for rolling behaviors to change direction. Therefore, a higher proportion of flip-twist behaviors observed in the current study may have been due to an individual's motivation to scent-mark rather than change their direction of movement. A future study could investigate how the width of a tunnel may influence the frequency of rolling behaviors that reverse orientation and upright turns. The frequency of these behaviors may also be affected by the size of the individual. The queen of colony 1200C gave birth to a litter of 18 pups two days after the study ended and her body was thus quite wide throughout the study. Unsurprisingly, she was the only individual never to be observed performing an upright turn, apparently because it was physically impossible for her to do so in a tube of that size. Perhaps larger individuals are more likely to use rolling behaviors than upright turns to change their direction of movement in narrow tunnels. In contrast, I have anecdotally observed outside of the current study young individuals that were

particularly small perform the flip-twist behavior despite the fact that the tube in which they were rolling was much wider than their body's width and even wider than their body's length. In other words, even very small individuals that would least likely have trouble performing an upright turn have been seen performing flip-twist rolling behaviors, suggesting the existence of an alternative function to that of changing the direction of movement. I propose that the flip-twist behavior originally evolved to function in changing the direction of movement in the confines of a narrow tunnel and still serves this purpose in certain contexts, and that this behavior was subsequently coopted as an exaptation (sensu Gould & Vrba, 1982) to function in scent-marking. It is therefore possible that at least some subset of the flip-twist rolling behaviors observed in the current study were for the purpose of changing the direction of movement. The current study was designed to increase the motivation of subjects to scent-mark by having a clean stimulus tube condition. Future studies could increase the motivation to reverse orientation and change direction of movement to see how the relative frequencies of rolling behaviors differ. In a forthcoming study, naked mole-rats were isolated in short tubes capped at both ends, motivating them to alternate between digging at the end caps in order to escape isolation and return to the colony. It is predicted that final analysis will reveal a high frequency of upright turns and rolling behaviors that result in reverse orientation while the frequency of rolling behaviors that maintain orientation will be nearly zero.

Although the current study describes four different rolling behaviors, the flip-twist, the flip-situp, the twist-twist, and the twist-situp, it may be more appropriate to

consider these behaviors separately as sequences of their component behaviors: the flip, the situp, and the twist. Perhaps the first behavior in the sequence serves a purpose independent of the second behavior in the sequence.

The initial flip or twist may be alternate methods for the individual to place their dorsum on the floor for the purposes of scent-marking; perhaps flips are preferred to twists because the dorsum of the individual seemingly more forcefully lands on the tunnel floor, possibly leaving a higher amount of odorous chemicals behind while scent-marking. Alternatively, the twist may function in scent-marking while the flip may function in changing the direction of movement because the forward flexion of the spine during a flip is more conducive to reversing orientation than is an upright turn which would require lateral flexion and may be physically impossible in a narrow tunnel. However, when the two rolling behaviors that begin with a flip were analyzed together or separately, their frequencies were significantly greater in the clean than the dirty stimulus condition, a finding which was predicted if the function of this behavior is scent-marking and not for changing the direction of movement. As for the two rolling behaviors that being with a twist, despite the fact that the frequency of the total of these two behaviors were about twice as common in the clean than the dirty stimulus tube condition, this difference was not significant. This is possibly due in part to the rarity of these two twist-first behaviors and that too few individuals performed them. Further study is warranted before ruling out twist-first rolling behaviors as functioning in scent-marking.

In the second part of the two-part sequence of behaviors that comprise rolling

behaviors, the twist or situp may simply be alternate methods for the individual to return to the upright position, perhaps depending on the direction in which the individual wants to go next. Under the condition of advanced pregnancy, it is also possible that situps are physically impossible and twists serve as a viable alternative. If a flip followed by a twist only functions for changing the direction of movement, the less common flip followed by situp may indicate that the individual changed their mind about reversing orientation halfway. Given the high frequency of flip-situp behaviors, this explanation seems unlikely. If a twist followed by a second twist only functions in scent-marking, a twist followed by a situp may simply reflect rare instances when a scent-marker decided to reverse orientation while on the floor before getting up. If the second part of the sequence, whether twist or situp, functions to restore the individual to the upright position and does not reflect the direction the individual wants to go, then perhaps those rolling behaviors that end with a situp were less common than those that end with a twist because, as it seems, situps require more energy to restore upright orientation.

Additional lines of evidence mentioned in the introduction further suggest that rolling behaviors that result in reverse orientation do not always function to change the direction of movement, and these should be further investigated. For example, the flip-twist rolling behavior, which seems more adaptive for reversing orientation in a tight tunnel, has been observed in voluminous nest boxes and tunnel joints that preclude such a need for this behavior. When visiting the laboratory of Dr. Vera Gorbanova at the University of Rochester, I observed a naked mole-rat perform three flip-twist

behaviors in two large chambers within 40 s punctuated by running through tubes connecting these chambers. It is clear that in such instances, rolling behaviors are not meant to function in changing the direction of movement.

A forthcoming study is investigating an entire colony's tunnel system to see all the locations where and at what relative frequencies the different types of rolling behaviors occur; perhaps certain rolling behaviors are more likely to occur in tunnels than in boxes or tube joints. Additionally, sometimes multiple flip-twist behaviors occur in rapid succession over a short distance, resulting in the individual maintaining their original orientation, and these bouts of flip-twist behaviors may shortly proceed or be succeeded by upright turns, which seemingly could have been executed in the first place. It seems unlikely that this is indicative of fickleness and more likely that these behaviors were not meant for the purpose of changing the direction of movement. The data from current study could be reanalyzed for bouts of rolling behaviors to better quantify this phenomenon.

What is the odor source of the scent-mark?

Neither scent glands nor exudate are apparent on the dorsal surface of naked mole-rats, so what is the odor source being deposited by rolling behaviors that function in scent-marking? Naked mole-rats lack sweat glands (Tucker, 1981) and, being hairless, also lack the sebaceous glands associated with hair follicles (Daly & Buffenstein, 1998). However, naked mole-rats shed a fair amount of dry skin dander, and I propose that this may be the odor source that is deposited by rolling behaviors.

Naked mole-rats produce a large amount of dry dead skin on their body surface, despite conditions of very high humidity (3 weeks of >85% relative humidity; F. R. Castelli, unpublished data). When colony tunnel systems are disassembled for routine cleaning, particles can often be shook out of tubes and an accumulation of dead skin (confirmed by microscopic inspection) can be found at the tunnel joints. *Chapter 2* discusses additional odor sources that naked mole-rats may use in olfactory communication, but it is dry skin dander that is the most likely candidate substance that could be spread from the naked mole-rats dorsal surface to the floor during a rolling behavior. A future study could coat the inside of a clean tube with collected dry skin dander and see if this elicits rolling behaviors similar to the dirty stimulus tube condition of the current study.

Scent-marking and olfactory communication

To confirm that scent-marks left by naked mole-rats performing rolling behaviors function in olfactory communication, future studies should investigate how these scent-marks act as signals to receivers by examining how receivers respond to them and how such responses may convey fitness benefits to the signalers. For example, odor deposited by rolling behaviors may signal a colony's territorial claim to a tunnel directed at members of foreign colonies. Naked mole-rats are highly xenophobic and it has been shown in the laboratory that they will violently attack conspecific intruders that break into their tunnel system (Lacey & Sherman, 1991; O'Riain & Jarvis, 1997). Perhaps by rolling in tunnels, individuals are trying to spread

their colony's odor so that foreigners are discouraged from entering. Individuals in the current study may therefore have been motivated to perform rolling behaviors in the clean stimulus tube condition to contribute to the accumulation of their colony's odor signature, thus following the rule, "scent-mark wherever odor from any member of my colony is lacking." However, it is unknown how likely colonies in the wild may encounter each other.

Alternatively, odor deposited by rolling behaviors may serve as a signal directed at fellow colony members. Naked mole-rats form dominance hierarchies (*Chapter 2*; Clarke & Faulkes, 1997, 1998; Schieffelin & Sherman, 1995), and in *Chapter 2* I have demonstrated that naked mole-rats can discriminate between the odors of two other members of their colony based on the dominance rank of odor donors. It remains to be seen if this ability to discriminate is based on individual recognition or some simpler cue. I speculate that rolling behaviors that function in scent-marking may communicate dominance by the pattern of deposition of scent-marks. Specifically, individuals may be trying to spread their personal identifying odor around the colony in as many places as possible. The very act of doing this is energetically costly and may serve as an honest signal of an individual's dominance and fighting prowess. Additionally, or alternatively, maximizing the spread of one's odor may communicate an individual's continued presence in the colony which may serve to keep subordinate individuals suppressed (see *Chapter 2* for additional discussion). Therefore, in the current study, subjects may have been following the rule, "scent-mark wherever odor from myself is absent." A forthcoming study was

designed to distinguish between this rule and the rule mentioned above, "scent-mark wherever odor from any member of the colony is lacking." Each half of the tunnel system was prepared with opposing stimulus odor conditions in three small colonies of two naked mole-rats. If the rule is to mark wherever any naked mole-rat odor is absent, then rolling behaviors should occur more frequently in the clean half than the half scented by the subject's fellow colony member. If the rule is to mark wherever the subject's personal odor is absent, then the rolling behaviors should in both halves at equal frequency.

The dominance hierarchies of the colonies used in the current study are unknown, but breeders are usually the most dominant individuals of their sex in each colony (*Chapter 2*; Clarke & Faulkes, 1997, 1998; Schieffelin & Sherman, 1995), so data from breeders in the current study provide some anecdotal evidence that scent-marking is related to dominance. In their respective colonies, the breeder male of colony 1200E showed the highest frequency of complete rolling behaviors and the breeder male of colony 1200C showed the highest number of rolling behaviors that began with a twist, but the breeder male of colony 1200B-2 only ever showed flip-twist behaviors and at a moderate frequency. The queens of colonies 1200B-2 and 1200E showed the third highest frequencies of complete rolling behaviors in their colonies, and the queen of colony 1200C unsurprisingly performed the lowest frequency of rolling behaviors in her colony, likely due to her state of advanced pregnancy. The current study only focused on rolling behaviors that occurred in the stimulus tube. A forthcoming study simultaneously observing the entire colony's

tunnel system will provide a better measure for how dominance rank may correlate with the frequency of rolling behaviors.

Potentially related behaviors

One or more rolling behaviors observed in the current study may be related to what Lacey and colleagues (1991; p. 218) refer to as "wallowing": "A *wallowing* animal rubs its shoulders or flanks against the bottom or sides of the toilet box or a nearby tunnel immediately after urinating or defecating; sometimes a wallowing animal rolls onto its back (plate 8-3). Wallowing typically occurs in the toilet [latrine] area…" I suspect that this description is referring to rolling behaviors and what I more aptly term as "side dragging," leaning to the side while moving forward so that the shoulder or flank drags along the side wall of the tube. Sometimes an individual's side will make contact with the side wall of a tube if the individual is running forward and they seemingly slip causing the body to lean. However, other times this behavior seems absolutely intentional as the individual will switch between left and right sides as they drag along the length of the entire stimulus tube. This behavior may be another form of scent-marking as dragging their body along the wall of a tunnel may serve to remove dry skin dander from their body and deposit it along the tunnel. This would nicely compliment rolling behaviors that would deposit dry skin dander on the floor instead of the sides of tunnels.

I also suspect that the rolling and on back behaviors mentioned in the description of wallowing by Lacey and colleagues (1991) may have been

misinterpreted and that naked mole-rats do not actually wallow in urine and feces. As discussed in *Chapter 2*, I have several reasons for believing this, but in brief, I have not observed this behavior casually or in 24-hours of video footage of the latrine of one colony, and wallowing in urine and feces seems counterproductive to the act of sequestering waste in a latrine for good health and hygiene. Lacey and colleagues (1991) also suggest that the function of wallowing in the communal latrine is to acquire or reinforce the distinctive colony odor signature found there, but I believe that naked mole-rats are more interested in standing out as individuals within their colony than they are to homogenize their colony odor for signaling to individuals from foreign colonies that they are far less likely to encounter. When using a latrine in the laboratory, the soiled areas extend over time out of the latrine box and into the tube leading to the latrine box, apparently due to a buildup of waste from individuals relieving themselves only at the entrance of the latrine. I suspect that Lacey and colleagues defined soiled tubes leading to the latrine as part of the "toilet area" and observed somersaulting in these tubes but mistook it for wallowing.

Conclusions

The current study finds support for the *rolling as scent-marking* hypothesis that rolling behaviors of naked mole-rats function as a form of scent-marking in olfactory communication. Four different rolling behaviors were observed and scored, three of which were described for the first time in naked mole-rats. Since two of these rolling behaviors result in a reverse body orientation, it is also possible that in certain

contexts these behaviors function in changing the direction of movement in a narrow tunnel. Future studies that use more ecologically relevant dirt tunnels may suggest additional or alternative functions for rolling behaviors. No sex differences were observed in rolling behaviors, but other untested demographic variables may have influence. Additional studies are needed to better understand rolling behaviors including those that investigate the effects of other demographic and spatiotemporal factors. For example, future studies should investigate how rolling behaviors as forms of scent-marking may be related to a naked mole-rat's ability to discriminate between the odors of fellow colony members based on dominance of the odor donor (*Chapter 2*).

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CHAPTER 5

CONCLUSIONS

The goal of this dissertation was to expand our knowledge of olfactory communication in animals by asking to broad questions: “What behaviors are involved in communicating with odors?” and “what information is communicated via odor?” These questions were explored using two rodent species, a species of dwarf hamster (*Phodopus sungorus*) and the naked mole-rat (*Heterocephalus glaber*).

In *Chapter 2*, I tested the hypothesis that self-grooming functions in olfactory communication in the *Phodopus sungours* dwarf hamster by looking to see if this behavior is elicited by social stimuli and to see if the amount of elicitation is influenced by the type of the social stimulus. A repeated measures design was used in which male and female *Phodopus sungorus* dwarf hamsters were exposed to cotton nesting material scented by same-sex and opposite-sex conspecifics, as well as an unscented control, to look for differences in self-grooming. As predicted, subjects self-groomed more in response to conspecific odor than unscented controls. Specifically, self-grooming in response to opposite-sex odor was higher than same-sex odor and unscented controls, which did not differ from each other, suggesting that a communicative function for self-grooming is to attract a mate. This pattern of self-grooming was similar when the grooming of different body parts was analyzed separately. Consistent with these findings, subjects spent more time investigating opposite-sex odor than same-sex odor or unscented controls, and subjects also showed

a greater interest in communicating with the opposite-sex through scent-marking. Suggestions for further study were discussed.

In *Chapter 3*, I tested the hypothesis that naked mole-rats are able to discriminate between the odors of fellow colony members based on the dominance of odor donors. A repeated measures design was used in which subjects of three colonies of naked mole-rats were tested using a T-choice apparatus of which each stimulus arm contained the whole-body odor of one of two fellow colony members that differed in dominance rank and choice was measured by stimulus arm entry. Subjects were tested with several pairs of stimulus odor donors that varied in dominance status, sex, breeding status, and body weight, and subjects were analyzed separately according to sex, breeding status, absolute dominance rank, and relative dominance rank. The overall results were rather consistent: naked mole-rats have the ability to discriminate between the odors of fellow colony members based on dominance, subordinate odors were never preferred, and dominant odors were mostly preferred. Additional questions generated by these results were discussed, including the identity of the odor sources that convey dominance, how these odors convey dominance, how the ability to discriminate between odors based on dominance helps to maximize fitness, and why the odors of dominant donors are preferred.

In *Chapter 4*, I tested the hypothesis that rolling behaviors in naked mole-rats function as a form of scent-marking. A repeated measures design was used in which a plastic tube in each of three colonies was replaced on alternating days with a clean tube or a “dirty” tube that had been part of the colony tunnel system for 24 h prior, and

rolling behaviors and upright turns were scored for 2.75 h each day for 20 days. As predicted, for most analyses, rolling behaviors were significantly more frequent in the clean than the dirty stimulus tube condition likely because clean tubes should motivate more scent-marking behavior. Upright turns occurred much more frequently than rolling behaviors that reverse orientation, suggesting that they are the preferred method for doing so. Also as predicted, the frequency of upright turns did not differ between the clean and dirty stimulus tube conditions, indicating that the difference in rolling behaviors was not likely due to a difference in interest. Lastly, as predicted, subject sex did not have a significant effect on rolling behaviors or upright turns. Overall, the results lend support to the hypothesis that rolling behaviors function in olfactory communication as a form of scent-marking. Dry skin dander as a possible odor source deposited by rolling behaviors was discussed, as well as several avenues for future research.

Despite a bias in humans against the cognizance of olfactory communication in the natural world, the body of our scientific knowledge on this topic is growing. Nevertheless, there are many additional avenues of investigation that students of olfactory communication could pursue and many potential study organisms to choose from. Additional study of this phenomenon is highly warranted to improve our understanding of the proximate and ultimate causes of animal behavior.